ISSCR ANNUAL MEETING 2022
SAN FRANCISCO
15–18 JUNE 2022

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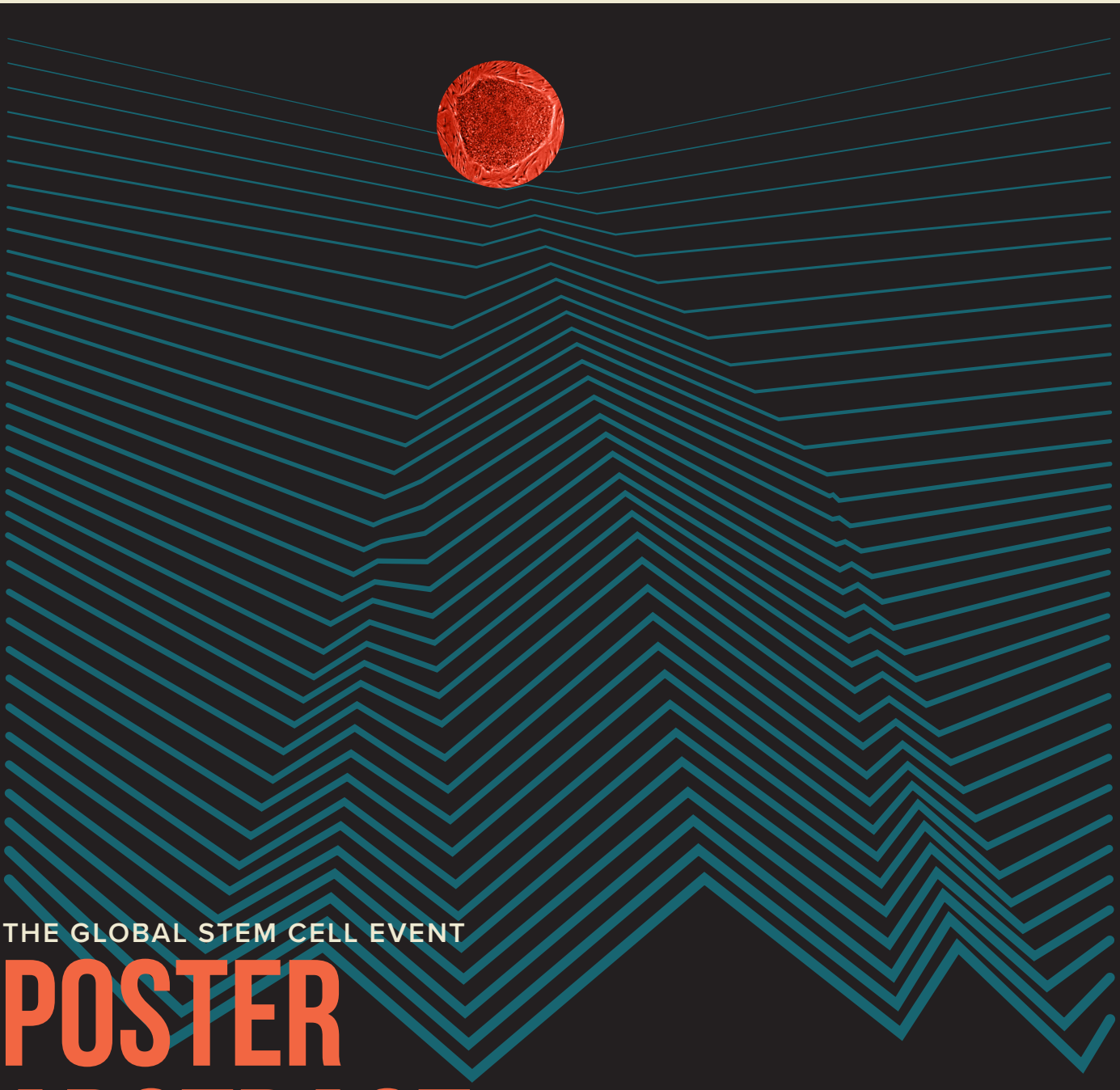
ISSCR 2022



THE GLOBAL STEM CELL EVENT

POSTER ABSTRACT GUIDE

20 YEARS OF EXCELLENCE

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POSTER SESSIONS

All times in PDT

WEDNESDAY, 15 JUNE, 2022

THURSDAY, 16 JUNE, 2022

FRIDAY, 17 JUNE, 2022

WEDNESDAY, 15 JUNE, 2022

POSTER SESSION I: ODD

6:30 PM – 7:30 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

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CROSSTALK BETWEEN HIPPO:YAP AND RA SIGNALING PATHWAYS SPECIFIES ATRIAL LINEAGES

Abraham, Elizabeth B. - *Center of Translational Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*
Stronati, Eleonora - *Cardiovascular Sciences, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*
Zubillaga, Mikel - *Cardiovascular Sciences, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*
Estaras, Conchi - *Cardiovascular Sciences, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*

Abstract: Heart development is controlled by a network of transcription factors that connect signaling pathways with genes for cell differentiation, patterning, and heart structure. Mutations in the cardiac gene network components are at the core of congenital heart defects (CHD). RA signaling is a vitamin A metabolite that regulates cardiogenesis by promoting the posterior specification of the cardiac progenitor cells (CPCs) in the Second Heart Field (pSHF) and the acquisition of atrial lineages. Defects in the differentiation trajectories of the SHF population give rise to CHD. Hence, excess or insufficient RA signaling causes developmental defects in the heart. However, the regulatory network controlling the specification of CPCs and cardiomyocyte (CM) identity is elusive. Intending to identify new factors involved in CPC specification in concert with RA, we applied a functional genomic approach in human Embryonic Stem Cell (hESC)-derived CPCs, at single-cell resolution. Our data identify the Hippo nuclear effectors YAP1:TEAD4 as potential cell-fate determinants of atrial CMs. YAP1 interacts with TEAD-DNA binding proteins to regulate CM proliferation during heart development, but their role in CPC differentiation is largely unknown. Our ongoing studies show that active YAP1: TEAD4 enhancers are required for the RA-transcriptional response during atrial commitment in CPCs. Our data show that YAP1 is recruited by RA signaling to atrial enhancers through RA-induced steroid nuclear receptor NR2F2, an essential atrial specifier. Our studies indicate that the RA-induced YAP1:TEAD enhancers are integrated with the cTF network of CPCs to regulate the specification of atrial lineages.

Keywords: cardiomyocyte specification, YAP and Retinoic Acid Signaling, NR2F2 and Coup-Tfii

TOPIC: EARLY EMBRYO

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SINGLE CELL ANALYSIS OF THE TRANSCRIPTIONAL DYNAMICS DURING EMBRYO DORMANCY REVEALS CRITICAL FUNCTION OF THE HIPPO PATHWAY IN THE MAINTENANCE OF PLURIPOTENT LINEAGE

Chen, Rui - *Embryonic Self-Organization Research Group, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*
Jeong, Hyun-Woo - *Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*
Fan, Rui - *Embryonic Self-Organization Research Group, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*
Adams, Ralf H. - *Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*
Bedzhov, Ivan - *Embryonic Self-Organization Research Group, Max Planck Institute for Molecular Biomedicine, Muenster, Germany*

Abstract: The implantation of the blastocyst into the uterine wall is a key step of the reproductive cycle, which mediates the connection of the embryo to the maternal tissues during the early stages of pregnancy. However, in some species, including the house mouse, this process can be put on hold, while the embryo falls into a reversible state of suspended animation, known as diapause. In contrast to the “normal” (transient) embryogenesis, which have been intensively studied, the embryonic diapause is still an extremely enigmatic state. Focusing on the pluripotent lineage, here we aimed to decipher the embryo-intrinsic mechanisms of embryo dormancy, using the mouse as a model system. Transcriptional analysis using single cell RNA-seq (scRNA-seq) approach revealed that the diapause is not a “stasis”, but instead is a dynamic state where gene expression is actively modulated. Moreover, we found that during diapause the epiblast also changes its tissue scale architecture, establishing epithelial rosette-like structure. The establishment of epithelial polarity in pluripotent lineage results in the inactivation of the Hippo signalling enabling the nuclear accumulation of the transcriptional co-factor Yap1. Although, the presence of Yap1 is not essential for the transient development of the epiblast, we found that deletion of Yap1 during diapause is critical for the maintenance of the pluripotent cells. Moreover, Yap1 function is also required for the efficient reactivation of the epiblast development upon exit of embryo dormancy. Modulating the Hippo pathway in vitro, in embryonic stem cells (ESCs), revealed the critical role of this cascade in keeping the programmed cell death at bay. Thus, our work elucidates an essential “cryptic” function of the Hippo/Yap1 signalling in the long-term maintenance of the pluripotent cells in vitro, in ESCs culture and in vivo, in diapause embryos.

Keywords: Embryonic diapause, Hippo/Yap1 signalling, pluripotent cells

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IMPDH FORMS THE CYTOOPHIDIUM DURING MOUSE EMBRYOGENESIS

Peng, Min - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*
Chang, Chia-Chun - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*

Liu, Ji-Long - *School of Life Science and Technology, ShanghaiTech University, Shanghai, China*
Sung, Li-Ying - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*

Abstract: Inosine monophosphate dehydrogenase (IMPDH) catalyzes the rate-limiting step of de novo guanine nucleotide biosynthesis. The levels of IMPDH RNA and protein were reported to be elevated robustly at the 4-cell stage of mouse embryos and onward. Moreover, the development of 4-cell stage embryos toward blastocysts was severely interrupted in the presence of IMPDH inhibitor, MPA, indicating an essential role of IMPDH at particular stages of embryogenesis. On the other hand, a membraneless sub-cellular structure of IMPDH, the cytoophidium, has been discovered in several types of mammalian cells. The formation of cytoophidium has been implicated to desensitize the enzymatic inhibitory effect by GTP binding, thereby accelerating GTP biosynthesis in physiological conditions. Herein we perform immunostain for IMPDH in preimplantation mouse embryos at different developmental stages and observe the presence of cytoophidium. The filamentation of IMPDH is hardly found in earlier stages but few cells in morulae. Blastocysts further display increased IMPDH cytoophidium formation, implying the association of cytoophidium assembly with IMPDH expression, and with cellular metabolism. Additionally, in postimplantation embryos from 7.5 to 17.5 dpc under normal physiological conditions, the IMPDH cytoophidium can be detected in multiple tissues, including olfactory epithelium, cerebrum, spinal cord, rib, thymus, intestine, liver, kidney, muscle, and skin. To further investigate the involvement of IMPDH cytoophidium in mouse embryo development, a previously identified IMPDH2 mutation, Y12C that disrupts polymerization, is applied to establish no-cytoophidium mutant mouse embryonic stem cells (ESCs) by CRISPR/Cas9 genome editing system. Wild-type and mutant ESCs are injected respectively into the cavity of blastocyst and injected embryos are transferred into the uterus of pseudopregnant females. Seventeen chimeric wild-type pups are born, with a term-development rate of 70.8%; however, none of the chimeric mutant offspring is generated, illustrating significantly compromised differentiation capacity of ESCs without cytoophidium-forming property. Our results reveal that cytoophidium assembly is not only a natural phenomenon but indispensable during mouse embryo development.

Funding Source: Research was supported by Ministry of Science and Technology, Taiwan, Grant Number 109-2313-B-002-003-MY2
Keywords: IMPDH, Cytoophidium, Embryo development

TOPIC: EPITHELIAL_GUT

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EPIGENETIC REPROGRAMMING OF INTESTINAL STEMNESS BY DIETARY ARACHIDONIC ACID

Garipcan, Aybuke - *Cancer Center, Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, USA*
Eskiocak, Onur - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Meydan, Cem - *Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA*
Yueh, Brian - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Shah, Vyom - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Chung, Charlie - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Papciak, Katherine - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Ozler, Kadir - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Bhalla, Kuhulika - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*
Alsudani, Habeeb - *Histology Core, CSHL, Cold Spring Harbor, NY, USA*
Habel, Jill - *LAR, CSHL, Cold Spring Harbor, NY, USA*
Rubino, Rachel - *LAR, CSHL, Cold Spring Harbor, NY, USA*
Stillman, Bruce - *CSHL, Cancer Center, Cold Spring Harbor, NY, USA*
Rivadeneira, David - *Surgery, Northwell Health, Woodbury, NY, USA*
Mason, Christopher - *Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA*
Meyer, Hannah - *Quantitative Biology, CSHL, Cold Spring Harbor, NY, USA*
Beyaz, Semir - *Cancer Center, CSHL, Cold Spring Harbor, NY, USA*

Abstract: Self-renewal and differentiation of intestinal stem cells (ISCs) are tightly regulated by niche-derived signals such as ligands, growth factors and cytokines emanating from neighboring Paneth cells, fibroblasts, enteric glia and immune cells around the intestinal crypt. Additionally, accumulating evidence posits that nutrients and metabolic pathways significantly influence cellular function and fate by signaling to transcription factors and altering epigenetic landscapes. While recent studies started to explore metabolic regulation of ISC activity through fatty acid (FA) oxidation, ketone body signaling, mitochondrial pyruvate metabolism and microbiome-derived metabolites, little is known about how nutrients and their metabolite derivatives influence ISC activity and cellular plasticity in the intestine by evoking epigenetic alterations. Here, through a screen of dietary FAs in mouse and human intestinal organoids, we characterize a subset of omega-6 family fatty acids that converge on arachidonic acid (AA) with robust stemness-enhancing effects. By utilizing isocaloric diets that vary in AA abundance, we demonstrate that AA promotes both homeostatic and damage-induced intestinal stem cell regeneration in vivo. Cross-species gene expression analysis reveals induction of conserved repair-associated stem cell reprogramming signatures in response to AA treatment. Using single-cell RNA sequencing (scRNA-seq), we identify AA-induced de novo stem cell states and dedifferentiation programs in vivo and in vitro. Mechanistically, dietary AA engenders production of epithelial prostaglandin E2 (PGE2), which activates PTGER4 – cAMP – PKA signaling axis to promote stemness in mice and humans. Finally, we find that AA evokes epigenetic reprogramming around stem cell regeneration-associated genes in a PTGER4-dependent manner. These findings demonstrate that dietary AA is a conserved promoter of stem cell regeneration that mimics the repair-response to tissue injury through PGE2-PTGER4 signaling and downstream epigenetic reprogramming.

Keywords: arachidonic acid, intestinal stem cells, epigenetic reprogramming

TOPIC: EPITHELIAL_SKIN

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REPROGRAMMING OF DISEASE-ASSOCIATED RENAL EPITHELIAL CELLS INTO INDUCED PLURIPOTENT STEM CELLS USING AN IMPROVED RNA-BASED METHOD

Frieman, Amy L. - *Dermatology, Gates Center for Regenerative Medicine, Aurora, CO, USA*
Diette, Nicole - *Biological and Physical Science, Keystone College, Scranton, PA, USA*
McGrath, Sean - *Pediatrics, University of Colorado, Aurora, CO, USA*
Kogut, Igor - *Dermatology, University of Colorado, Aurora, CO, USA*
Bilousova, Ganna - *Dermatology, University of Colorado, Aurora, CO, USA*

Abstract: Induced pluripotent stem cells (iPSCs) hold enormous potential for regenerative medicine and disease modeling. Common reprogrammed cell types include fibroblasts, keratinocytes, and blood cells. However, a major drawback to obtaining fibroblasts and keratinocytes from skin biopsies and blood cells from venipuncture is the potential risk for complications, especially in patients with skin and connective tissue disorders. Moreover, exposure to ultraviolet rays and high cell turnover rates increases the likelihood of problematic chromosomal abnormalities and mutations in these cell types. The collection of urine is a non-invasive method and can provide exfoliated renal epithelial cells (RECs), which are amenable to reprogramming. Current non-integrating methods of REC reprogramming utilize DNA plasmids or Sendai viral vectors. However, these methods are typically associated with elevated cell death, long and tedious regimens and low efficiency of reprogramming. Here, we report the successful reprogramming of healthy RECs and RECs derived from individuals with recessive dystrophic epidermolysis bullosa (RDEB) and Down Syndrome into iPSCs using a non-integrating RNA-based approach. This approach was adapted from our previously published high-efficiency RNA-based protocol for reprogramming fibroblasts into iPSCs by modifying cell culture conditions and REC transfection regimens. As a result, we developed a protocol that consistently generates clinically relevant iPSCs from RECs at high efficiency. The generated iPSCs are chromosomally stable, express pluripotency markers, and can be differentiated in vitro and in vivo. Thus, not only can our methodology consistently generate high quality iPSCs with little to no discomfort for patients, but it also possesses a tremendous potential for future translational and therapeutic applications.

Funding Source: DEBRA International, Linda Crnic Institute for Down Syndrome, and Gates Frontiers Fund
Keywords: Induced Pluripotent Stem Cells (iPSCs), RNA Reprogramming, Renal Epithelial Cells

TOPIC: GERMLINE

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EED IS REQUIRED FOR PRIMORDIAL GERM CELL DIFFERENTIATION IN THE EMBRYONIC GONAD

Lowe, Matthew - *MBIDP, University of California, Los Angeles (UCLA), CA, USA*
Yen, Ming-Ren - *Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan*
Hsu, Fei-man - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Gorgy, Isaac - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Chitiashvili, Tsotne - *Molecular Biology Institute, UCLA, Los Angeles, CA, USA*
Bernard, Matthew - *Molecular Biology Institute, UCLA, Los Angeles, CA, USA*
Hu, Zhongxun - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Hosohama, Linzi - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Hunt, Timothy - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Wamaitha, Sissy - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Chen, Pao-Yang - *Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan*
Clark, Amander - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*

Abstract: Primordial germ cells (PGCs) are the founding cells of the entire adult germline, and their proper differentiation is essential for the survival of the species. During mammalian PGC differentiation, major epigenetic reorganization occurs which is hypothesized to establish a regulatory landscape for sex-specific germ cell differentiation and gametogenesis. In both human and mouse PGCs, this reorganization includes the near complete removal of DNA methylation as well as a global enrichment of nuclear histone 3 lysine 27 trimethylation (H3K27me3). While DNA methylation has previously been shown to have a critical role in regulating the timing of PGC differentiation in mice, the role of H3K27me3 is less clear. To address this, we used the mouse model to create a PGC specific conditional knockout for Embryonic Ectoderm Development (EED), a critical protein in polycomb repressive complex 2 (PRC2) which catalyzes H3K27me3. Using imaging, FACS, bulk, and single cell RNA sequencing we found that EED is essential for regulating the timing of sex-specific PGC differentiation in both ovaries and testes as well as X chromosome decompensation in XY PGCs. Integrating H3K27me3 ChIP and bisulfite sequencing of mouse epiblast and PGCs, we identified a subset of intermediate to high GC content gametogenesis promoters with a uniquely poised repressive signature of high H3K27me3 and DNA methylation. Through Co-immunoprecipitation we detected an interaction between EED/PRC2 and DNMT1 in Epiblast-Like cells providing a mechanism for the establishment and/or maintenance of this signature from at least the epiblast stage of embryo development. Taken together, we propose that EED joins DNMT1 in regulating the timing of sex-specific PGC differentiation through the generation of a repressive H3K27me3/DNA methylation epigenetic mark at gametogenesis promoters. Results from these studies will be critical to advance

our understanding of mammalian PGC epigenetic regulation and to improve PGC differentiation in vitro from pluripotent stem cells.

Funding Source: NIH/NICHD 2 R01 HD058047, Ruth L. Kirschstein National Research Award GM007185, UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Training Program and the Rose Hill Foundation Scholarship
Keywords: PGCs, PRC2, Germline

TOPIC: HEMATOPOIETIC SYSTEM

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DISCOVERING CELL IDENTITY BY DATA INTEGRATION AND PROJECTION

Choi, Jarny - *Department of Anatomy and Physiology, University of Melbourne, Australia*
Rajab, Nadia - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Butcher, Suzy - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Angel, Paul - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Elahi, Zahra - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Wells, Christine - *Department of Anatomy and Physiology, The University of Melbourne, Australia*

Abstract: As we accumulate gene expression data at an unprecedented rate, discovering and predicting the transcriptional identity of a cell becomes a critical step in many downstream analyses. The problem is not simply a computational one of finding best clusters in high dimensional data. Since cells are assayed under different conditions and derived from different types of donors or other cell types, meaningful cell identity profiling which works beyond a single dataset needs to take these other features of the data properly. Furthermore, batch correction remains a challenge when separate datasets are merged together. An additional challenge is that most tools for cell identity profiling have been developed for bioinformaticians and it is difficult for a bench biologist to perform any analyses. At stemformatics.org, we have developed novel integrated atlases, which combine multiple datasets to create comprehensive transcriptional landscape of blood cells. Batch effect correction has already been done using a novel gene filtering strategy as well as careful sample annotation where we applied uniform terminology across all the datasets. This enables easy cross-dataset comparisons of cells. A key feature is that external data can be projected easily onto the atlas using online tools, empowering users to benchmark their own cells against the reference cells for rapid hypothesis generation. We have also developed a computational workflow called Sincast to project single cell data onto a Stemformatics atlas or other bulk references for cell identity prediction. Sincast uses either pseudo-bulk aggregation or zero imputation to make single cell data compatible with bulk data before projection is performed.

Keywords: Transcriptional profiling, Reference atlas, Benchmarking

TOPIC: KIDNEY

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LOCATING THE ORIGIN OF THE AMNIOTIC FLUID STEM CELLS ON A HUMAN FETAL KIDNEY SPATIAL TRANSCRIPTOMIC MAP

Beesley, Max A. - *Great Ormond Street Institute of Child Health, University College London, UK*
Xenakis, Theodoros - *Great Ormond Street Institute of Child Health, University College London, UK*
Martini, Paolo - *Molecular and Translational Medicine, University of Brescia, Italy*
Kunpalin, Yada - *Great Ormond Street Institute of Child Health, University College London, UK*
David, Anna - *EGA Institute for Women's Health, University College London, UK*
De Coppi, Paolo - *Great Ormond Street Institute of Child Health, University College London, UK*
Gerli, Mattia Francesco Maria - *Division of Surgery and Interventional Science, University College London, UK*

Abstract: Human amniotic fluid stem cells (AFSCs) are mesenchymal, multipotent stem cells. AFSCs are autologous to the fetus and are isolated through amniocenteses and amniodrainages, making them ideal for fetal/neonatal regenerative medicine. By comparing the AFSC bulk RNAseq gene expression profiles with a library of human mesenchymal cell lineages isolated from multiple fetal tissues, our group indicated that the AFSCs originate from the fetal kidney. We have now applied spatial transcriptomics to human fetal kidney samples (n=3, 20PCW) to investigate the precise anatomical site of origin of the AFSCs. To achieve this, we developed a novel analytical workflow which allowed us to overlay our bulk RNAseq expression profiles onto these ad hoc generated spatial transcriptomics data. As first step, we integrated a single-cell kidney dataset with our spatial transcriptomics data to increase its resolution to single-cell level. We then applied the NewWave R package to normalise the combined dataset composed of both our AFSC bulk RNAseq and high-resolution kidney spatial transcriptomics data. This normalisation enabled these samples to be analysed and graphically represented together. All AFSC lines analysed (n=8), clustered to the same renal anatomical region, which showed the greatest transcriptomic similarities to the AFSCs. This lends further support to our current model, which indicates that the AFSCs shed from the kidney epithelia and translocate to the amniotic fluid through the fetal urine. By identifying the precise cell population that gives rise to the AFSCs, we were able to investigate the transcriptomic changes leading to their formation. These findings are aiding the identification of novel markers for AFSC isolation, providing new insights on the developmental processes involved in their generation and improving our understanding of their identity.

Funding Source: UCL Wellcome ISSF Restarting Research Great Ormond Street Hospital Charity PhD Studentship Marie Skłodowska-Curie Fellowship NIHR GOSH BRC GOS ICH Institute Development Fund
Keywords: Amniotic Fluid Stem Cells, Spatial Transcriptomics, Fetal Kidney

TOPIC: MUSCULOSKELETAL

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SYSTEMATIC DISCOVERY OF STEM CELL FATE REGULATORS FOR PRECISION CANCER THERAPY

Chung, Mingyu - *Neurology, Stanford University, Mountain View, CA, USA*
Wheeler, Joshua - *Pathology and Neuropathology, Stanford University, Stanford, CA, USA*
Krishnan, Ananya - *Neurology, Stanford University, Palo Alto, CA, USA*
Ishak, Heather - *Neurology, Stanford University, Palo Alto, CA, USA*
Chang, Howard - *Dermatology and Genetics, Stanford University, Stanford, CA, USA*
Rando, Thomas - *Molecular, Cell, and Developmental Biology, University of California Los Angeles, CA, USA*

Abstract: A major goal in medicine is to control stem cell fate specification, yet its regulatory logic remains poorly understood. In rhabdomyosarcomas (RMS), tumor propagation often depends on epigenetic dysregulation of the myogenic regulatory factors (MRF) MyoD and MyoG, raising the possibility that stemness can be modulated via lineage-specific TFs. Despite these discoveries, RMS is heterogeneous, and our ability to predict pathway sensitivity in individual cases remains extremely limited. We aim to discover regulatory pathways controlling MyoD and MyoG in order to direct stemness as well as predict targeted interventions for individual cases of RMS. We performed an image-based phenotypic drug screen of over 1300 compounds involved in epigenetic regulation on primary mouse muscle progenitors, measuring MyoD and MyoG expression along with late differentiation-associated myotube fusion. Strikingly, the targets implicated from our screen encompass many pathways reported across diverse RMS instances, suggesting that the key MRF regulatory pathways in primary progenitors are the same ones that RMS subtypes hijack. Our screen further implicates numerous targets not previously reported in RMS but which are upregulated in RMS data sets. We are now phenotypically drug screening diverse RMS lines, and we will test the impact on tumorigenicity for a subset of drugs representing specific phenotypic patterns to predict in vivo sensitivity from in vitro phenotype. Finally, we will integrate epigenomic and transcriptomic profiles of RMS and progenitor cells to discover markers that predict phenotypic response and tumorigenic sensitivity. The discovery of better predictive markers for precision therapy in RMS would constitute a major medical advance and highlight the potential of targeting stem cell fate specification in other hierarchical cancers and their associated stem cells.

Funding Source: Funding provided by F32GM139260 (MC) and Stanford Physician Scientist Faculty Incubator (JRW).
Keywords: Myogenesis, Cancer, Epigenetics

TOPIC: NEURAL

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P53 BOTH INHIBITS AND PROMOTES CELLULAR REPROGRAMMING

Beitz, Adam M. - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
Lende, Brittany - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
Galloway, Kate - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*

Abstract: The tumor-suppressor protein p53 maintains genomic integrity in response to genomic insults, earning it the moniker of “guardian of the genome.” In reprogramming, p53 serves as a barrier. High levels of p53 induce cell cycle arrest or apoptosis, which limit reprogramming efficiency. Inclusion of mutant p53 in the reprograming cocktail induces up to a 100-fold increase in induced motor neurons. Curiously, this p53 mutant enables rapidly proliferating cells to accumulate wild-type p53. Knockdown of wild-type p53 in the presence of this mutant reduces reprogramming efficiency, indicating an indispensable role for wild-type p53 in reprogramming that our mutant p53 cannot fulfill. How then does wild-type p53 both limit and promote reprogramming? Using mathematical modeling to examine the p53 response to DNA damage, we find that repression of p53’s transactivation activity by mutant p53 can directly lead to accumulation of wild-type p53. Further, we demonstrate using our model that increased levels of p53 may enable reprogramming cells to tolerate higher rates of transcription. We hypothesize that p53 mitigates DNA damage induced by hypertranscription through interaction with topoisomerase I (Top1). We model this interaction as a p53 concentration-dependent inhibition of DNA damage resulting from increased transcription rates. To test our hypothesis, we have generated p53 mutants with altered tetramerization and Top1-interaction domains. We observe specific domain mutants result in different patterns of localization and proliferation. Through characterization of the mutant proteins, we are defining the specific protein-protein and protein-DNA interactions that allow p53 to promote high efficiency reprogramming. Together, our data suggest a dual role for p53 in cell-fate transitions that functions as a band-pass filter to restrict transitions. Understanding how p53 promotes cellular plasticity may help to identify processes that promote reprogramming and that can be enhanced without oncogenic mutants. Our observations also implicate a role for p53 in oncogenic transitions and may help to identify specific interactions that could be interrupted to limit cancerous transformations.

Funding Source: A.M.B. is supported by the National Science Foundation Graduate Research Fellowship Program under grant No. 1745302.
Keywords: p53, motor neurons, reprogramming barriers

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A POOLED VIRAL TRANSCRIPTION FACTOR LIBRARY APPROACH TO DISSECT NEURONAL DIVERSITY

Mazzara, Pietro G. - *Genetics and Development, Columbia University, New York, NY, USA*
Baldwin, Kristin - *Genetics and Development, Columbia University, New York, NY, USA*
Buckley, Tyler - *Genetics and Development, Columbia University,*

New York, NY, USA
Lilion, Hanna - *Genetics and Development, Columbia University, New York, NY, USA*
Yoney, Anna - *Genetics and Development, Columbia University, New York, NY, USA*

Abstract: Investigating human neuronal diversity is one of the challenges of today's neuroscience. Many neurologic diseases are linked to widely expressed genes that have effects only on rare and specific neuronal subtypes. Due to the post-mitotic nature of neurons, disease etiology and treatments have historically been explored using animal models that can poorly replicate human symptoms and progression. Using reprogramming and pluripotent stem cells it is possible to produce many human subtypes of interest for disease, such as peripheral sensory neurons, motor neurons and dopaminergic neurons. Yet, for the majority of cell types in the brain, we lack methods to identify or produce them in vitro. To overcome this problem, we have established a somatic cell reprogramming strategy to generate the whole plethora of neuronal subtypes by screening a pooled lentiviral library of transcription factors (TFs) expressed in neural lineages and involved in neuronal specification and maturation. Using a new genome edited induced pluripotent stem cell (iPSC) SNAP25-P2A-tdTomato reporter cell line, single tdTomato positive induced neuronal cells have been isolated. These induced neurons show strong morphological diversity (unipolar, bipolar and multipolar neuronal morphology) among them. Moreover, our preliminary analyses, show strong TFs diversity between cells and expand the list of TFs able to generate iNs in vitro. Linking the TF input to the transcriptional output signatures will expand the reprogramming toolbox and may also shed light on mechanisms underlying the generation of neuronal diversity in humans.

Funding Source: Supported by NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation
Keywords: Direct Reprogramming, Neurons, Screening

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SINGLE-CELL RNA SEQUENCING OF THE DEVELOPING HUMAN MENINGES

Vinsland, Elin - *Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*
Linnarsson, Sten - *Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*

Abstract: The vertebrate central nervous system is encased by the meninges, which consists of three connective tissue layers called the pia-, arachnoid-, and dura mater. The meninges attach the brain to the skull, provide mechanical protection and blood, and have space through which cerebrospinal fluid travels around the brain. Increasing evidence also shows an active involvement of the meninges in brain development. Furthermore, meningiomas are the most common primary intra-cranial tumors and originate from the meninges. Since molecular drivers for growth, invasion and transformation are mostly unknown, no specific biomarkers or targeted therapies exist. However, the cellular composition and development of the meningeal layers is poorly understood, and no single-cell RNA sequencing has been performed on the developing human meninges. In this study, we aim to (1) characterize cellular heterogeneity during early development of the human meninges using single-cell RNA sequencing, and (2) compare it with published data from meningioma resections to investigate if aspects of meningioma biology can be explained by developmental programs. We generated an atlas of 170,000 single cells from the prenatal human meninges between postconceptional weeks (PCW) 5.5-13.5. We identify 363 clusters, covering cellular heterogeneity within mesenchymal (fibroblasts and pericytes)-, vascular endothelial-, epithelial-, neural (progenitors, glia, neurons)-, neural crest-, and hematopoietic lineages. Almost 10% of the cells are cycling, and the onset of gene expression associated with arachnoid and dura development occurs at PCW 9 onwards. Some other findings include gene expression gradients between fibroblast clusters, pericytes, and smooth muscle cells, a common progenitor for epithelial and neural lineages, and the appearance of microglia already at PCW 5.5. Comparing the embryonic meninges with meningioma tumors showed that the major cell classes and the percentage of cycling cells are similar. Further analysis may give insight into whether meningioma grades correlate with developmental maturity, help identify the cell of origin and progression markers. Our transcriptomic atlas gives novel insight into early human meningeal development, and can help characterize meningioma biology on a cellular and molecular level.

Funding Source: Swedish Foundation for Strategic Research (SB16-0065), Knut and Alice Wallenberg Foundation (2018.0172 and 2018.0220), Erling-Persson Foundation (HDCA), and the European Union (BRAINTIME) to S.L.
Keywords: Meninges, Embryonic, Meningioma

TOPIC: PANCREAS

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MYCL-MEDIATED REPROGRMMING EXPANDS PANCREATIC INSULIN-PRODUCING CELLS

Hirano, Michitada - *Institute of Medical Science, The University of Tokyo, Japan*
Yamada, Yasuhiro - *Institute of Medical Science, The University of Tokyo, Japan*

Abstract: β-cell proliferation declines with age and mature β-cells have a limited capacity for regeneration, which predisposes towards diabetes. Here, we show that, of the MYC family members, Mycl plays a key role in proliferation of pancreatic endocrine cells. Genetic ablation of Mycl caused a reduction in the proliferation of pancreatic endocrine cells in neonatal mice. By contrast, the expression of Mycl in adult mice stimulated the proliferation of pancreatic islet cells, resulting in the expansion of pancreatic islets, without tumour formation in other organs. Single-cell RNA sequencing and genetic tracing experiments revealed that Mycl expression had a transcriptional signature that is associated with immature proliferating endocrine cells and stimulated the division of adult hormone-expressing cells. The expanded hormone-expressing cells stopped proliferating, but persisted after the withdrawal of Mycl expression. Remarkably, a subset of the expanded α-cells gave rise to insulin-producing cells after this withdrawal. Moreover, transient Mycl expression in vivo was sufficient to normalise the hyperglycaemia of mice with diabetes. In vitro expression of Mycl similarly provoked active replication in mature hormone-expressing islet cells, even in those from aged mice. Finally, we show that MYCL stimulates the division of human adult cadaveric islet cells. Our results demonstrate that the induction of Mycl alone elicits proliferation in adult islet cells and expands the functional β-cell population, both in vivo and in vitro, which may provide a regenerative strategy for β-cells.

Keywords: Reprogramming, Regeneration, MYCL

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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VIABILITY OF BOVINE FETAL MSC POST CRYOPRESERVATION DIFFERS DEPENDING ON THE TISSUE OF ORIGIN AND CRYOPROTECTANT SOLUTION

Oyarzo, Rudy - *Escuela de Graduados Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile*
Jaramillo, Catherinne - *Instituto de Farmacología y Morfofisiología, Universidad Austral de Chile, Valdivia, Chile*
Ditzel, Claudio - *Instituto de Farmacología y Morfofisiología, Universidad Austral de Chile, Valdivia, Chile*
Bahamonde, Javiera - *Instituto de Farmacología y Morfofisiología, Universidad Austral de Chile, Valdivia, Chile*

Abstract: The study of mesenchymal stem cells (MSC) and their therapeutic potential in domestic species has become quite relevant, especially in pets or high-level sport animals. However, in cattle the information available is rather limited, even though MSC have been postulated as a good therapeutic alternative for important diseases like mastitis. One of the limitations for the development of MSC-based therapies is the high variability among MSC. It has been proven that even MSC coming from different tissues from the same individual could not behave in the same way when faced with certain stimuli such as cryopreservation, broadly used to preserve MSC. For this reason, it is extremely important to develop tailored protocols for each cell type. The aim of our research was to evaluate the viability of bovine fetal MSC from adipose tissue (AT-MSC), bone marrow (BM-MSC) and placental tissue (PT-MSC), after cryopreservation with 4 cryoprotectant solutions. Cells were kept under standard culture conditions until passage 2, when they were divided into 5 groups: the Control group was not cryopreserved and experimental groups were cryopreserved with 10% dimethyl sulfoxide (DMSO, D10), 10% ethylene glycol (EG, E10), 5% DMSO and 5% trehalose (D5T5), or 5% EG and 5% trehalose (E5T5). After cryopreservation, viability was evaluated at 0, 24 and 48 hours. MSC from different tissue sources did not respond equally to cryopreservation, but the differences were not evident until 24 hrs after being thawed. At this time point, AT-MSC showed higher viability than PT-MSC in all groups except E10, and AT-MSC also presented higher viability than BM-MSC for E5T5. Nevertheless, all MSCs were able to recover after 48 hrs and the differences disappeared. Regarding the cryoprotectant solutions, all significant differences within tissues were detected immediately post-thawing. E5T5 demonstrated the worse performance in all cell types, and D10 exhibited the best performance in AT-MSC, but there were no differences between D10, E10, and D5T5 in BM-MSC and PT-MSC. In conclusion, the viability of bovine fetal MSC post cryopreservation differs depending on the tissue of origin and cryoprotectant solution used. Further studies are needed to determine the potential effect of cryoprotectant solutions on therapeutic related MSC properties for each MSC type.

Funding Source: This research was funded by the Chilean Nacional Agency for Research and Development (ANID), Project FONDECYT 11180681.
Keywords: cryoprotectants, bovine fetal MSC, post-thawing viability

TOPIC: PLURIPOTENT STEM CELLS

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TISSUE ARCHITECTURE INFLUENCES BRAIN ORGANOID IDENTITY AND DEVELOPMENTAL TRAJECTORIES

Chiaradia, Ilaria - *Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UK*
Imaz-Rosshandler, Ivan - *Cell Biology, MRC LMB, Cambridge, UK*
Nilges, Benedikt - *Creative Campus Monheim, Resolve Biosciences GmbH, Monheim am Rhein, Germany*
Das, Richa - *Creative Campus Monheim, Resolve Biosciences GmbH, Monheim am Rhein, Germany*
Kashikar, Nachiket - *Creative Campus Monheim, Resolve Biosciences GmbH, Monheim am Rhein, Germany*
Lancaster, Madeline - *Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UK*

Abstract: Organs are the ensemble of different cell types in a complex architectural milieu. It is well-known that progression through fate decisions sets up the complex cellular makeup and architecture of an organ, but how that same architecture may impact on cell fate is less clear. We sought to examine this by taking advantage of the unique capabilities of organoids as a tractable in vitro model to interrogate how fate and form interact during organ development. We found that patterning signals early on drive cell fate and affect tissue morphology, just as in vivo. We next focused on the reverse question, how does tissue architecture shape tissue identity? To this aim, we perturbed organoid morphology using various complementary approaches and analysed the transcriptome as a whole and in its tissue context, through the integration of single nuclei RNA sequencing and spatial transcriptomics. Mechanically redistributing cells in a random spatial conformation resulted in profound changes in tissue identity, including loss of certain neuron types and neural progenitors, but enrichment of tissue signalling centres – such as cortical hem. This points to a plasticity of the tissue in re-establishing key organizing cues. This, combined with more subtle changes to tissue architecture provided a spectrum of different organoid morphologies. Scoring based on morphological features appeared to be a better predictor of organoid quality and reliability in mimicking in vivo human fetal brain development than the specific protocol used. Furthermore, single cell spatial transcriptomics revealed that organoids with poor tissue architecture displayed altered developmental trajectories. Neural progenitors and neuronal markers were intermingled in time and space. Our findings suggest that not only does the temporal progression of fate decisions control spatial architecture of the tissue, but space also governs time. Cells that lack proper spatial coordinates also fail to undergo the proper temporal progression of events.

Keywords: Organoid, Morphology, Transcriptome

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STRATEGIES TO AVOID IMPRINTING DEFECTS IN INDUCED PLURIPOTENT STEM CELLS

Da Rocha, Simao T. - *Stem Cell Engineering Research Group, Instituto de Bioengenharia E Biociências, Instituto Superior Técnico, Uni, Porto-Salvo, Portugal*
Arez, Maria - *SCERG - Stem Cell Engineering Research Group, Instituto de Bioengenharia e Biociências, Instituto Superior Técnico, Universidade de Lisboa, Porto-Salvo, Portugal*

Eckersley-Maslin, Melanie - *Cancer Biology & Therapeutics Program, Peter MacCallum Cancer Centre, Melbourne, Australia*
Klobucar, Tajda - *Ule's lab, National Institute of Chemistry, Ljubliana, Slovenia*
Von Gilsa Lopes, João - *CEDOC, NOVA Medical School, Lisbon, Portugal*
Krueger, Felix - *Bioinformatics Group, Babraham Institute, Cambridge, UK*
Mupo, Annalisa - *Epigenetics Programme, Babraham Institute, Cambridge, UK*
Raposo, Ana Cláudia - *Instituto de Bioengenharia e Biociências, Instituto Superior Técnico, Universidade de Lisboa, Porto-Salvo, Portugal*
Gendrel, Anne-Valeire - *Sérgio de Almeida's Lab, Instituto de Medicina Molecular Joao Lobo Antunes, Lisboa, Portugal*
Bernardes de Jesus, Bruno - *IBIMED, Universidade de Aveiro, Aveiro, Portugal*

Abstract: Reprogramming of somatic cells into induced Pluripotent Stem Cells (iPSCs) is a major leap towards personalized approaches to disease modelling and cell-replacement therapies. However, we still lack the ability to fully control the epigenetic status of iPSCs, which is a major hurdle for their downstream applications. A sensible indicator for epigenetic fidelity is genomic imprinting, a phenomenon dependent on DNA methylation, which is frequently perturbed in mouse and human iPSCs by yet unidentified reasons. By using a reprogramming murine system with hybrid donor cells, we conducted a thorough imprinting analysis using allele-specific IMPLICON in multiple female and male mouse iPSCs (miPSCs) generated under different culture conditions. Our results show that imprinting defects are remarkably common in miPSCs causing dysregulation of the typical monoallelic expression of imprinted genes. Interestingly, the nature of imprinting defects depends on the sex of the donor cell and their respective response to culture conditions. Under serum-free conditions, male miPSCs show global hypomethylation at imprinted regions, whereas in serum conditions show focal hypermethylation at specific loci. In contrast, female miPSCs always exhibit hypomethylation defects regardless of culture conditions. These imprinting defects are more severe than the global changes in DNA methylation, highlighting the sensitivity of imprinting loci to current iPSC generation protocols. With this knowledge, we are trying to improve the reprogramming process to generate miPSCs devoid of imprinting errors. We will present this novel data and discuss the implications of our results for human iPSC derivation. Our results are essential for future development of reprogramming protocols for the derivation of epigenetically faithful iPSCs for translational and clinical applications.

Funding Source: Work in S.T.d.R.'s team is supported by FCT/MCTES, Portugal (PTDC/BIA-MOL/29320/2017) and LaCaixa Foundation, Spain (HR20-00322). S.T.d.R. has a CEECUIND/01234/207 assistant research contract from FCT/MCTES, Portugal.
Keywords: Genomic Imprinting, induced pluripotent stem cells (iPSCs), DNA methylation

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PLURIPOTENCY STATE MODULATES APICOBASAL POLARITY IN EMBRYONIC STEM CELLS

Shi, Yu - *Molecular Biosciences, The University of Texas, Austin, TX, USA*
Kopparapu, Nitya - *Molecular Biosciences, University of Texas at Austin, TX, USA*
Dickinson, Dan - *Molecular Biosciences, University of Texas at Austin, TX, USA*

Abstract: Apicobasal polarity - creating molecularly distinct apical and basolateral plasma membrane domains - is essential for gastrulation and body planning, and its disruption causes embryonic lethality. The cells in the mouse epiblast establish apicobasal polarity as they exit naïve pluripotency, resulting in an asymmetric distribution of cellular components, including cell junctions, trafficking proteins, and membrane-binding proteins. Despite the temporal coordination, it remains unclear whether and how polarity establishment and naïve pluripotency exit are linked. To address this, we established live imaging procedures to monitor the polarization process in real-time while manipulating the pluripotency level. To trace and control the polarization process, we used 3D spheroid cultures of mouse embryonic stem cells (ESCs) as a model. ESCs in the Naïve medium generate an unorganized structure, while ESCs in Naïve Pluripotency Exit medium establish apicobasal polarity. Our data show how ESCs in Exit medium establish polarity by generating a single apical domain marked by localization of tight junctions, sialomucins (Podocalyxin), and Par polarity proteins. Polarity establishment can be reversed by switching polarized cells back to Naïve medium. Live imaging of ESCs expressing Podocalyxin-mNG reveals the dynamic process of apical domain assembly and disassembly during polarity establishment and polarity loss. Altogether, our results reveal the crosstalk between polarity and pluripotency state and provide insights into how mammalian cells establish cell polarity, which contributes to finding new therapies for polarity-associated diseases.

Funding Source: This work is supported by funding from the Cancer Prevention and Research Institute of Texas (RR170054).
Keywords: Cell polarity, Stem cell, Live imaging

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NME7 INDUCES HOMOGENEOUS OCT4 POSITIVE XAXA POPULATION OF NAÏVE STEM CELLS WHILE BETA-CATENIN INDUCES A SUB-POPULATION OF OCT4 POSITIVE XAXA NAÏVE-LIKE CELLS WHICH INCREASES DIFFERENTIATION POTENTIAL

Bamdad, Cynthia C. - *Stem Cell Group, Minerva Biotechnologies, Waltham, MA, USA*
Yi, Kevin - *Stem Cell Group, Minerva Biotechnologies, Waltham, MA, USA*
Nash, Jac-Leen - *Stem Cell Group, Minerva Biotechnologies, Waltham, MA, USA*
Carter, Mark - *Stem Cell Group, Minerva Biotechnologies, Waltham, MA, USA*
Page, Danica - *Stem Cell Group, Minerva Biotechnologies, Waltham, MA, USA*
Smagghe, Benoit - *Stem and CAR-T, Minerva Biotechnologies,*

Waltham, MA, USA
Stewart, Andrew - *Stem and CAR-T, Minerva Biotechnologies, Waltham, MA, USA*

Abstract: The literature is replete with conflicting reports as to whether the Wnt/β-catenin pathway induces human stem cell differentiation or pluripotency. Recently scientists showed that human stem cells expressing low levels of active β-catenin preferentially differentiate down neuroectoderm lineage, whereas cells expressing high levels favor differentiation to mesendoderm. However, these results appear to contradict two other studies, where researchers improved differentiation to both neuroectoderm and mesoderm by increasing levels of active β-catenin at the start of differentiation. Here, we show that stem cells cultured in medium containing naïve growth factor, NME7, express the highest levels of active β-catenin, yet readily differentiate into neuro-ectoderm cells and mes-endoderm, without lineage preference. This raised the interesting question of whether activation of the Wnt/β-catenin pathway could itself play a role in maintaining or inducing a naïve-like state. To answer that question, the β-catenin agonist WNT3A was added to stem cells, in the absence of any other growth factors, including those present in adhesion layers. Surprisingly, WNT3A induced the concurrent emergence of two segregated populations: an OCT4+, XaXa naïve-like population and an OCT4- population. This finding could explain the apparently conflicting reports in the literature as to whether β-catenin induces pluripotency or differentiation, while raising a new set of intriguing questions. Notably, does the naïve-like sub-population, devoid of cell fate decisions, contribute to an increased differentiation potential of the overall population? Conversely, could the OCT4- cells differentiate better because they are poised to differentiate? To address these questions, we compared the differentiation of primed state stem cells, with or without pre-treatment with WNT3A, to that of naïve state stem cells. Pre-treatment of primed state stem cells with WNT3A improved their differentiation potential, whereas the addition of WNT3A to naïve stem cells had no effect. In each case, the differentiation of the naïve cells was superior to the primed state cells, even after pre-treatment with WNT3A, which is consistent with the idea that the improved differentiation is due to the sub-population of the WNT3A induced naïve-like cells.

Keywords: naïve, beta-catenin, differentiation potential

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IKBA IS A NEW REGULATOR OF PLURIPOTENCY, MESOSDERMAL FATE ACQUISITION AND HEMATOPOIETIC DIFFERENTIATION

Palma, Luis G. - *Stem Cells and Cancer, Hospital Del Mar Research Institute, Barcelona, Spain*
Álvarez-Villanueva, Daniel - *Stem Cells and Cancer, Hospital del Mar Research Institute, Barcelona, Spain*
Iglesias, Arnau - *Stem Cells and Cancer, Hospital del Mar Research Institute, Josep Carreras Leukaemia Research Institute, Barcelona, Spain*
Thambyrajah, Roshana - *Stem Cells and Cancer, Hospital del Mar Research Institute, Josep Carreras Leukaemia Research Institute, Barcelona, Spain*
Rodríguez, Virginia - *Stem Cell biology, Developmental Leukemia and Immunotherapy, Carreras Leukaemia Research Institute, Barcelona, Spain*
Bueno, Clara - *Stem Cell Biology, Developmental Leukemia and Immunotherapy, Carreras Leukaemia Research Institute, Barcelona, Spain*
Menendez, Pablo - *Stem Cell Biology, Developmental Leukemia and Immunotherapy, Carreras Leukaemia Research Institute, Barcelona, Spain*
Espinosa, Lluis - *Stem Cells and Cancer, Hospital del Mar Research Institute, Barcelona, Spain*
Bigas, Anna - *Stem Cells and Cancer, Hospital del Mar Research Institute, Josep Carreras Leukaemia Research Institute, Barcelona, Spain*

Abstract: Besides inhibition of NF-κB signaling, IκBα has a critical role in skin homeostasis and intestinal differentiation, by regulating cell fate acquisition and stem cell homeostasis. Our group identified an ancestral chromatin function for IκBα, conserved in C. Elegans and Drosophila, that together with polycomb proteins was regulating cell fate determination. Within this context, we have investigated whether IκBα plays a role in mouse embryonic stem cell (mESCs) pluripotency and differentiation. We generated IκBα-/- ES cells and compared to WT differentiation towards mesoderm/endoderm/hematopoietic fates using embryoid bodies (EBs)-based cultures. IκBα-/- cells acquired lower levels of mesendodermal markers and showed a great reduction of Flk1+ hemo-mesodermal progenitors. Flk1+ IκBα-/- cells were non-functional with a compromised ability of producing different types of blood progenitors. We found that mESCs lacking IκBα cannot switch off the pluripotency network as demonstrated by the upregulation of pluripotency genes at different times of EB-differentiation, the presence of pluripotency proteins (NANOG and OCT3/4) and high percentage of alkaline phosphatase positive cells in EBs after day 9 of differentiation compared to IκBα+/+ counterparts. Transcriptome analysis of mESCs demonstrated that IκBα-/- cells display a dysregulation of Polycomb Repressive Complex 1 (PRC1) and Complex 2 (PRC2) targets genes, whereas no clear evidence in NF-κB-target genes overactivation is observed. Finally, in reprogramming experiments using E14.5 IκBα+/+ and IκBα -/- mouse embryonic fibroblasts (MEFs), we observed a similar reprogramming efficiency in both MEFs, but a more robust pluripotent phenotype in the colonies from IκBα-/- MEFs. Altogether, these results support a model in which IκBα is regulating the exit from pluripotency through modulating the binding of polycomb proteins to their target genes and allowing the differentiation of ES cells.

Funding Source: This work has been supported by Ministerio de Ciencia e Innovación (PID2019-104695RB-I00, SAF2016-75613-R), Generalitat de Catalunya (2017 SGR 135 and 2019 FI-B 00151/2020 FI_B1 00130) and Departament de Salut (SLT002/16/00299).
Keywords: Pluripotency, NF-KB pathway, Hematopoiesis

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ENDOGENOUS NUCLEIC ACID GENERATION IN BAT SPECIES

Singh, Arun A. - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine, Mount Sinai, New York, NY, USA*
Déjosez, Marion - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Parejo, Carlos - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Gray, Jonathan - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Qin, Yiren - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Xu, Hui - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Zwaka, Thomas - *Cell, Developmental and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract: Bats have a number of enviable biological adaptations, only recently disclosed, which elevate them among mammals. Species can exhibit hummingbird-like metabolic rates and human-scale lifespans, with no apparent incidence of diabetes, atherosclerosis, or cancer. Disavowal of age-related pathologies, oxidative stress, and apparent resistance to DNA damage are remarkable features of bats that are still poorly understood. However, sustainable models of bat biology are still undergoing characterization. In assessing induced pluripotent stem cells derived from wild specimens of Rhinophilus ferrumequinum and Myotis myotis, deviations from conventional mammalian biology can be noted in ordinary phases of the cell cycle. Flow cytometric and microscopic analyses disclose apparent parallel proliferations of nucleic acids. These entities do not incorporate thymidine and are evidently distinct from the normal process of synthesis. Yet they remain cell cycle associated, and potentially this phenomenon shows species-specificity in the order Chiroptera. As well among the unique and important capacities bats have demonstrated, is a millennia-long imbrication with RNA viruses. Observations and standardization of bat iPSCs may thus further understanding of bat fecundity as viral hosts, progenitors and of attendant molecular mechanisms.

Keywords: bats, induced pluripotent stem cells, cell cycle

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DEUBIQUITINASE OTUD5 IDENTIFIED AS NOVEL REGULATOR OF HUMAN PLURIPOTENCY THROUGH MULTI-LINEAGE CRISPR SCREENS

Rosen, Bess P. - *BCMB Allied Program, Weill Cornell Medical School, Brooklyn, NY, USA*
Cho, Hyunwoo - *Developmental Biology, Memorial Sloan Kettering, New York, NY, USA*
Pan, Heng - *Proteomics and Microchemistry Core Facility, Memorial Sloan Kettering, New York, NY, USA*
Li, Qing - *In Vivo Rewriting Candidate Development, Tessera Therapeutics, Cambridge, MA, USA*
Hendrickson, Ronald - *Proteomics and Microchemistry Core Facility, Memorial Sloan Kettering, New York, NY, USA*
Sidoli, Simone - *Department of Biochemistry, Albert Einstein College of Medicine, New York, NY, USA*
Huangfu, Danwei - *Developmental Biology, Memorial Sloan Kettering, New York, NY, USA*

Abstract: Pluripotency in the early human embryo is not a stable self-renewing state but is transient, dissolving quickly into the three germ layers. However, most genetic screens interrogating human pluripotency have been performed in stable, self-renewing, hESC and hiPSC lines. In contrast, we have performed CRISPR screens explicitly investigating the dissolution of pluripotency as it occurs in development, directly into primitive endoderm and ectoderm lineages. We used multiple coding genome scale CRISPR screens to assay parallel differentiation and maintenance conditions in hESCs with the goal of both identifying novel regulators of pluripotency, and distinguishing regulators of pluripotency and self-renewal. Two screens utilized an OCT4-GFP reporter to assay the loss of pluripotency in the context of neuroectoderm or definitive endoderm differentiation. In addition two self-renewal screens were performed by dropout in the context of normal E8 hESC maintenance condition, or prolonged growth in the absence of TGFβ or FGF2. The deubiquitinase OTUD5 was identified as a novel regulator of the dissolution of human pluripotency in all 4 screening conditions. Subsequent generation of knockout lines has shown that loss of OTUD5 sensitizes hESCs to challenges to pluripotency — such as removal of growth factors, addition of differentiation cues, or passaging in harsh conditions — irrespective of lineage context. Further mass-spectrometry experiments have suggested OTUD5 regulates chromatin modifiers already known to have a role in the transition and commitment between the pluripotent state and lineage specification. The discovery of OTUD5 deubiqtuinase as a regulator of pluripotency has revealed a novel regulatory network fine-tuning the exit of pluripotency. Additionally these screens serve as a valuable resource for identifying gene networks that regulate pluripotency, and its loss, in a context dependent manner relevant to human development.

Keywords: CRISPR screen, pluripotency, lineage specification

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CELL COMPETITION CONSTITUTES A BARRIER FOR INTERSPECIES CHIMERISM

Hu, Yingying - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Zheng, Canbin - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Sakurai, Masahiro - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Pinzon-Arteaga, Carlos - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Wei, Yulei - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Yu, Leqian - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Wu, Jun - *Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Abstract: Pluripotent stem cells (PSCs) provide an invaluable in vitro model to study early mammalian development and hold great potential in revolutionizing regenerative medicine. More recently, PSC-derived interspecies chimeras have provided a means to generate complex three-dimensional tissues in vivo, which may help overcome the world-wide shortage of donor organs for transplantation. Although adult interspecies chimeras with extensive donor cell contribution have been achieved among several rodent species, low levels of chimeric contribution from donor PSCs were detected in evolutionarily distant host species, even at early developmental stages. Cell competition, the process of eliminating viable but “less fit” neighbor cells, has gained increasing recognition as an evolutionarily conserved mechanism for development, tissue homeostasis, and stem cell maintenance. 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 interspecies pluripotent cell competition improves chimerism between evolutionary distant species. 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 or rhesus cells in early mouse embryos. Analysis of bulk RNA-seq and scRNA-seq provided us more clues for dissecting underlying mechanisms of interspecies PSC competition.

Funding Source: NYSCF-Robertson Stem Cell Investigator Award NIH/NICHD R01 HD103627-01A1 CPRIT RR170076
Keywords: Cell competition, Pluripotent stem cell, Interspecies chimerism

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CAPRIN1 LINKS EMBRYONIC STEM CELL DIFFERENTIATION WITH RNA METABOLISM

Viegas, Juliane O. - *Genetics, The Hebrew University, Jerusalem, Israel*
Azad, Gajendra - *Zoology, Patna University, Patna, India*
Lv, Yuan - *Integrative Biology, Guangzhou Institutes of Biomedicine and Health, Guangzhou, China*
Paltiel, Tal - *Genetics, The Hebrew University of Jerusalem, Israel*
Pattabiraman, Sundararaghavan - *Zoology, Patna University, Patna, India*
Park, Jung - *School of Biological Sciences, Nanyang Technological University, Nanyang Drive, Singapore*
Kaganovich, Daniel - *Experimental Neurodegeneration, University Medical Center Göttingen, Waldweg, Germany*
Sze, Siu - *School of Biological Sciences, Nanyang Technological University, Nanyang Drive, Singapore*
Rabani, Michal - *Genetics, The Hebrew University of Jerusalem, Israel*
Esteban, Miguel - *Integrative Biology, Guangzhou Institutes of Biomedicine and Health, Guangzhou, China*
Meshorer, Eran - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Embryonic stem cells (ESCs) are self-renewing and pluripotent. In recent years, factors controlling pluripotency, mostly nuclear, have been identified. To reveal non-nuclear regulators of ESCs, we screened an endogenously-labelled fluorescent fusion-protein library in mouse ESCs. One of the more compelling hits was the cell cycle-associated protein, CAPRIN1. CAPRIN1, a Stress Granule (SG) component, exhibited a strikingly cyclical localization pattern in sync with mitosis, and localized to SGs, in response to stress. CAPRIN1 knockout had little effect in ESCs, but dramatically skewed differentiation and gene expression programs. RIP-seq and SLAM-seq revealed that CAPRIN1 associates with, and promotes the degradation of thousands of RNA transcripts. CAPRIN1 interactome identified XRN2 as the likely ribonuclease. Upon early differentiation or stress, XRN2 colocalizes with CAPRIN1 inside SGs in a CAPRIN1-dependent manner. We propose that CAPRIN1 regulates an RNA degradation pathway operating during early ESC differentiation, eliminating undesired spuriously transcribed transcripts in ESCs.

Funding Source: EpiSyStem Network, Marie Curie Actions
Keywords: ESC differentiation, CAPRIN1, RNA stability

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ACCELERATING THE USE OF INDUCED PLURIPOTENT STEM CELLS IN PERSONALIZED MEDICINE AND DRUG DISCOVERY

Hartman, Jessica - *Product Applications, Cell Microsystems, Durham, NC, USA*
Stern, Allysa - *Product Applications, Cell Microsystems, Durham, NC, USA*
Land, Lexi - *Product Applications, Cell Microsystems, Durham, NC, USA*

Abstract: Induced pluripotent stem cells (iPSCs) are an invaluable tool for generating multiple cell types from individual patients. However, the workflows associated with growing, CRISPR gene editing, and differentiating iPSCs in 2D and 3D culture are inefficient and low-throughput, costly, time-consuming, and manually labor-intensive. We demonstrated that thousands of single human iPSCs can be screened per array without the use of feeder cells, as the arrays can be coated with a variety of iPSC compatible matrices, including Matrigel and laminin. iPSCs were serially imaged on the CellRaft AIR System to confirm viability, growth, and pluripotency via on array live Tra1-60 staining. CellRafts containing single-cell derived iPSC clones were isolated with the AIR System without manual manipulation or enzymatic dissociation (>90% efficiency), and clones maintained pluripotency off array. In addition, human iPSCs cultured in extracellular matrix on the CytoSort Array formed hundreds of individually segregated 3D organoids that were isolated intact into 96 well plates with >90% efficiency and no loss of 3D structure or viability. The iPSC-derived organoids could also be differentiated on-array into multiple organoid types, including choroid plexus and kidney. To determine whether stem cell-derived organoids could be used for drug screening off array, 3D mouse hepatic progenitor organoids (StemCell Technologies) were grown on array, and organoids were analyzed and isolated based on size using the Off-the-AIR software. Single organoid drug toxicity screening was performed on either heterogenous organoids (diameter >50µm) or homogenous organoids (diameter 300-500µm) using a 6-point dose curve of the known hepatotoxicant Acetaminophen (0.0008-2.5mM, n=5 organoids/dose). The results demonstrated that heterogeneity in organoid size yielded an uninterpretable dose curve, whereas the homogenous population allowed for calculation of an ED50% (0.6mM). The development of 2D and 3D stem cell workflows on an automated platform has the potential to increase the utility, ease, and throughput of these workflows, thereby accelerating the use of iPSCs in personalized medicine and drug discovery.

Funding Source: This work was funded by NIGMS SBIR 1R43GM143978-01.
Keywords: iPSC-derived organoids, iPSC cloning, drug screening

TOPIC: EARLY EMBRYO

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UNCOVERING THE REGULATORY MECHANISM OF TRANSPOSABLE ELEMENTS IN CELL FATE DETERMINATION DURING HUMAN EARLY EMBRYOGENESIS

Xiang, Xinyu - *University of Edinburgh Institute, Zhejiang University, Haining, China*
Xue, Ziwei - *Zhejiang University-University of Edinburgh Institute, Zhejiang University, Haining, China*
Guo, Yixin - *Zhejiang University-University of Edinburgh Institute, Zhejiang University, Haining, China*
Clark, Amander - *Department of Molecular Cell and Developmental Biology, University of California, Los Angeles, Los Angeles, CA, USA*
Liu, Wanlu - *Zhejiang University-University of Edinburgh Institute, Zhejiang University, Haining, China*

Abstract: Early human embryos originate from a fertilized egg which undergoes cleavage and ultimately formation of the blastocyst which then implants into the wall of a uterus to establish a pregnancy. Following implantation, the post-implantation human embryo will undergo significant morphological changes including an elaborate change in extra embryonic cell fate and morphology, amniotic cavity, yolk sac cavity and bilaminar disc formation as well as gastrulation to generate the somatic embryonic progenitors of the body. In recent years it has now appreciated that Transposable Elements (TEs) are transcriptionally active during human early embryo development where they serve essential roles. Notably some of these TEs are found only in primates and in some cases only in hominoids, suggesting that with evolution from old world primates, the gene regulatory network that regulates early human embryo development may have diverged. To address this, we used single cell RNA-Seq datasets of human and nonhuman primate (cynomolgus monkeys) early embryo development. From this resource, we developed a deep learning-based autoencoder model to decode the unique TE expression patterns and underlying roles in human early embryogenesis that differed from nonhuman primate models. Through integrated dimension reduction analysis of gene and TE transcriptome at single cell level, a more sophisticated cell atlas was depicted, and a systematic gene-TE interaction network was established.

Funding Source: NSFC (National, 32170551; Zhejiang Provincial LQ20C060004), FRFCU 2021QN81016 and Alibaba Cloud.
Keywords: Embryogenesis, Transposable Elements, Deep Learning

TOPIC: EPITHELIAL_SKIN

703

HIPSC-DERIVED SKIN ORGANOIDS AS TOOLS FOR DISEASE MODELING: CHARACTERIZATION OF THE EPIDERMAL-DERMAL JUNCTION

Ramovs, Veronika - *Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Janssen, Hans - *Electron Microscopy Facility, Netherlands Cancer Institute, Amsterdam, Netherlands*
Fuentes, Ignacia - *Fundación DEBRA Chile, Santiago, Chile*
Pitaval, Amandine - *IRIG-BIOMICS, CEA/INSERM/University of Grenoble Alpes, Grenoble, Netherlands*
Rachidi, Walid - *IRIG-BIOMICS, CEA/INSERM/University of Grenoble Alpes, Grenoble, Netherlands*
Chuva de Sousa Lopes, Susana - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Freund, Christian - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Gidrol, Xavier - *IRIG-BIOMICS, CEA/INSERM/University of Grenoble Alpes, Grenoble, France*
Mummery, Christine - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Raymond, Karine - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Human induced pluripotent stem cell (hiPSC)-derived hair-bearing skin organoids offer exciting new possibilities for modelling diseases like epidermolysis bullosa. These inherited diseases affect 1 in 30,000 people worldwide and result from perturbed expression and/or structure of components of the epidermal-dermal junction, the interface between basal keratinocytes of the epidermis and stroma of the dermis. To establish whether hiPSC-derived skin organoids might be able to capture salient features of epidermolysis bullosa, it is thus important to understand the structure and developmental stage of their epidermal-dermal junction. To this end, we successfully generated hair-bearing skin organoids from three independent hiPSC lines following the multistep protocol recently developed by Koehler's research group and thoroughly characterized their epidermal-dermal junction. Using immunofluorescence and electron microscopy, we showed that basal keratinocytes in organoids adhere to laminin-332 and type IV collagen-rich basement membrane via type I hemidesmosomes and integrin β1-based adhesion complexes. Importantly, we demonstrated that epidermal-dermal junctions in organoids are almost devoid of type VII collagen, a fibril that mediates anchorage of the epidermis to dermis. This indicates that further maturation is required to take full advantage of skin organoids as disease model for some forms of epidermolysis bullosa, in particular those caused by mutations in the COL7A1 gene.

Funding Source: This work was supported by DEBRA Austria and the Novo Nordisk Foundation (NNF21CC0073729). KR is Chargé de Recherche at the Institut National de la Santé et de la Recherche Médicale (INSERM).
Keywords: skin organoids, epidermal-dermal junction, type VII collagen

TOPIC: HEMATOPOIETIC SYSTEM

705

MAPPING HUMAN MEGAKARYOCYTE PROGENITOR DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS

Lawrence, Moyra - *Centre for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan*
Shahsavari, Arash - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Bornelöv, Susanne - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Moreau, Thomas - *Cambridge Stem Cell Institute, University of Cambridge, UK*
McDonald, Rebecca - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Vallance, Thomas - *Cambridge Stem Cell Institute, University of*

Cambridge, UK
Kania, Katarzyna - *Cancer Research UK Cambridge Institute, University of Cambridge, UK*
Paramor, Maike - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Baye, James - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Perrin, Marion - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Steindel, Maike - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Jimenez-Gomez, Paula - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Penfold, Christopher - *Department of Physiology, Development and Neuroscience, University of Cambridge, UK*
Mohorianu, Irina - *Cambridge Stem Cell Institute, University of Cambridge, UK*
Ghevaert, Cedric - *Cambridge Stem Cell Institute, University of Cambridge, UK*

Abstract: Platelet deficiency, known as thrombocytopenia, can cause haemorrhage and is treated with platelet transfusions. We previously developed a system for the production of platelet precursor cells, called megakaryocytes (MKs), from induced pluripotent stem cells (iPSCs). These cultures can be maintained for >100 days, implying culture renewal by megakaryocyte progenitors (MKPs). However, it is unclear whether the MKP state in vitro mirrors the state in vivo. Additionally, MKPs cannot currently be purified using conventional surface markers. We performed single-cell RNA sequencing throughout in vitro differentiation from iPSCs to MKs and mapped each state to its equivalent in vivo, revealing the accelerated formation of MKs without the generation of haematopoietic stem and progenitor cells. We then used machine learning approaches to identify five surface markers which can reproducibly purify MKPs from both iPSC- and cord blood haematopoietic stem cell-derived MK cultures. Purifying MKPs allowed us an insight into the transcriptional and epigenetic profiles of these cells which are usually deep in the bone marrow. Furthermore, it allowed us to characterise the increased telomere length of MKPs in comparison to surrounding MKs. Lastly, we performed culture optimisation, increasing MKP production. This optimisation feeds into process analysis already carried out on the protocol at Good Manufacturing Practice (GMP) Grade to allow higher yields of clinical grade platelets for transfusion. Together, this study has mapped parallels between the MKP states in vivo and in vitro and allowed the purification of MKPs as well as the investigation of their transcriptional and epigenetic state, accelerating the progress of in vitro–derived transfusion products toward the clinic.

Funding Source: U.K. Regenerative Medicine Platform, Pluripotent Stem Cell and Engineered Cell Hub (MR/R015724/1). Wellcome Trust (203151/Z/16/Z) to the Cambridge Stem Cell Institute.
Keywords: Megakaryocytes, Single-cell RNA sequencing, Progenitor cells

TOPIC: NEURAL

707

THE ROLE OF TRACE AMINE RECEPTORS IN DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO DOPAMINERGIC NEURONS

Katolikova, Nataliia V. - *Institute of Translational Biomedicine, Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia*
Shafranskaya, Daria - *Center for Algorithmic Biotechnology, Center for Algorithmic Biotechnology, Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia*
Prjibelski, Andrey - *Center for Algorithmic Biotechnology, Center for Algorithmic Biotechnology, Institute of Translational Biomedicine, Saint-Petersburg State University, Saint-Petersburg, Russia*
Mor, Mikael - *Institute of Translational Biomedicine, Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia*
Zaytseva, Anastasia - *Almazov National Medical Research Centre, Sechenov Institute of Evolutionary Physiology and Biochemistry, Almazov National Medical Research Centre, Sechenov Institute of Evolutionary Physiology and Biochemistry, Saint-Petersburg, Russia*
Vaganova, Anastasia - *Institute of Translational Biomedicine, Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia*
Efimova, Evgeniya - *Institute of Translational Biomedicine, Institute of Translational Biomedicine Saint-Petersburg State University, Saint-Petersburg, Russia*
Gainetdinov, Raul - *Institute of Translational Biomedicine, Institute of Translational Biomedicine Saint-Petersburg State University, Pirogov Clinic of High Medical Technologies, Saint-Petersburg State University, Saint-Petersburg, Russia*

Abstract: Trace amines (TA) and their receptors (trace amine-associated receptors, TAARs), are known to play role in regulation of movement, nutrition, attention, and mood. Changes in TA level and TAARs system are associated with a spectrum of neurological diseases such as schizophrenia, drug abuse and others. But their influence on the development of neuronal system is unknown. Previously we have shown that knockout of TAAR5 in mice leads to increased number of dopaminergic neurons in Substantia nigra and to increased number of doublecortin-positive cells in dentate gyrus. The aim of this study was to find the impact of TAARs on differentiation of human induced pluripotent stem cells (IPS cells) to dopaminergic neurons. The RNA-seq data from open resources was used. RNA-seq data of WTSIi004-A and WTSIi032-A cell lines from European Collection of Authenticated Cell Cultures, dataset EGAD0000100615 from European Genome-phenome Archive, single cell RNA-seq data ERP121676 from European Nucleotide Archive, GSE118723, GSE118412, GSE132758, GSE86654 was obtained to analyze the expression of TAARs in IPS cell lines and in different stages of differentiation of human IPS cells to dopaminergic neurons. The TPM coverage characteristic was used as a metric to estimate the quantity. Then three IPS cell lines were differentiated to dopaminergic neurons according to the protocol, described in Doi et al. (2014). The cell identity was confirmed by immunofluorescence, patch clump and HPLC analysis. RNA was obtained at day 0, 12, 28 and 53 of differentiation and analyzed using TagMan real-time PCR assay. The expression of TAARs at

different stages of differentiation of human IPS cell lines to dopaminergic neurons was found. It has a trend to increase compared with undifferentiated IPS cell lines, but the level of expression is low. TAAR5 expression was found in the vast majority of data that have been analyzed. Its expression can be found mainly at 53 day of differentiation, but in some lines it can be found at early stages. Expression of other TAARs (TAAR1, TAAR2, TAAR6 and TAAR8) was found in different stages in some cell lines also, but it is not repeated from study to study. So, the changes in expression of TAARs during the differentiation process was shown, but to make a conclusion about its impact the research should be continued.

Funding Source: This work was supported by the Russian Science Foundation grant 21-75-20062
Keywords: trace amine receptors (TAARs), human induced pluripotent stem cells (human IPS cells), dopaminergic neurons

TOPIC: PANCREAS

709

DEFINING REQUIREMENTS FOR THE ACTIVATION OF ADULT HUMAN PANCREATIC EXOCRINE CELLS IN A 3D SUSPENSION CULTURE

Zook, Heather N. - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*
Quijano, Janine - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*
Ortiz, Jose - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*
Donohue, Cecile - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*
Lopez, Kassandra - *Irell & Manella Graduate School of Biological Sciences, City of Hope, Duarte, CA, USA*
Jou, Kevin - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*
Li, Wendong - *Department of Surgery, City of Hope, Duarte, CA, USA*
Garcia, Isaac - *Arthur Riggs Diabetes & Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Montero, Enrique - *Arthur Riggs Diabetes & Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Ku, Teresa - *Translational Research and Cellular Therapeutics, City of Hope, Duarte, CA, USA*

Abstract: Damage to pancreatic exocrine cells occurs during the pathogenesis of pancreatitis and pancreatic ductal adenocarcinoma (PDAC). However, the early events that initiate the activation of these cells and the contribution of the microenvironment to their growth, function, and regeneration are still not well defined. This study aimed to devise novel culture systems to study the requirements of growth factors and extracellular matrix (ECM) proteins on human exocrine cell survival, activation, and expansion. After islets were isolated, the remaining human pancreatic exocrine tissues were dissociated into single-cell suspension, cryopreserved, thawed, and plated into a 3D suspension (spheroid) culture composed of DMEM/F12, knockout serum replacement, and six exogenous growth factors. Using single-cell RNA-sequencing, we identified exocrine cells resembling pancreatic duct cells that survived over the 7-day culture, with very few displaying an active cell cycle. To determine whether ECM proteins enhance cell cycle activity, Matrigel was added at a low concentration (5% vol/vol) to avoid the confounding effects of stiffness brought by higher concentrations. Compared to the control, adding Matrigel significantly increased the total cell number and the number of organoid-forming progenitor-like cells, indicating enhanced cellular expansion over the 7-day culture. Immunofluorescence analysis revealed a significant increase in proliferating (EdU+KI67+) and daughter cells (EdU+KI67-) and a significant decrease in apoptosis (CC3+). Additionally, bulk mRNA-sequencing revealed upregulation of metabolism, protein synthesis, and cell survival pathways and downregulation of inflammatory and apoptotic pathways. To investigate which ECM protein may bind to exocrine cells, a chip containing 36 combinations of different ECM proteins was used. We found that cells preferentially bound to combinations that included collagen IV, collagen VI, and/or vitronectin. Subsequently, cells cultured with recombinant collagen IV, but not collagen VI or vitronectin, significantly increased ATP production, suggesting its role in the metabolic activation of exocrine cells. Taken together, these results suggest that ECM proteins play a critical role in triggering human exocrine cell activation and expansion.

Funding Source: This work was supported by The Wanek Family Project for Type 1 Diabetes.
Keywords: Human pancreatic exocrine cells, 3D spheroid culture, Extracellular Matrix Proteins

TOPIC: PLURIPOTENT STEM CELLS

711

PHENOTYPIC ABNORMALITIES IN HUMAN PLURIPOTENT STEM CELLS UPON ACTIVATION OF REVERSE TETRACYCLINE TRANSACTIVATOR

Kamaldinov, Timothy - *Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*
Zhang, Linyi - *Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*
Shin, Hyunsu - *Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*
McGrath, Erica - *Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*
Ye, Zhaohui - *Gene Transfer and Immunogenicity Branch, Division of Cellular and Gene Therapies, Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*

Abstract: Tetracycline-controlled transcriptional activation is a widely used method for achieving inducible transgene expression. The tight control of the expression levels made reverse tetracycline transactivator (rtTA)-based Tet-On systems an attractive tool to study gene function, as well as for the in situ expression of the components from the synthetic biology toolbox. The evolution of the Tet-On system led to the development of improved transactivators, promoters and response elements that significantly enhanced the efficiency and specificity of the system, while minimizing the background levels of induction in the absence of tetracycline and its derivatives. However, the potential off-target effects of this system on cellular phenotypes have

not been thoroughly investigated in human stem cells such as iPSCs. Here, we report that the latest generations of rtTA-based Tet-On systems can result in partial loss of stem cell identity upon doxycycline induction. Among observed abnormalities are the loss of common morphological features associated with undifferentiated iPSCs, such as colony compactness and large nucleus-to-cytoplasm ratio, and the reduction in the protein expression levels of canonical pluripotency transcription factors and surface markers, such as Oct4, Nanog, SOX2, TRA-1-81 and SSEA-3. Transcriptome-wide changes were observed by RNA-seq starting at 24 hours of induction, including downregulation of most known pluripotency-associated genes. Critically, the phenotypic abnormalities were observed at all induction-permissive doxycycline concentrations; however, greater doses exacerbate the effects. Ongoing research investigates the effects of different Tet-on variants on stem cell identity, and mechanisms underlying the observed phenotypic changes. Collectively, our work demonstrates that tetracycline-controlled transcriptional activation may lead to undesirable off-target effects in iPSCs and further optimization of the variants and induction conditions may be necessary to ensure its proper use in stem cell research.

Keywords: reverse tetracycline transactivator, off-target effects, induced pluripotent stem cells

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CPG-FREE INSERTION-BASED CPG ISLAND METHYLATION RESPONSE (CIMR) IS RESTRICTED TO PRIMED PLURIPOTENCY

Lizhar, Elizabeth M. - *Arthur Riggs Diabetes and Metabolism Research Institute (AR DMRI), City of Hope, Duarte, CA, USA*
Shokrani, Alireza - *AR DMRI, City of Hope, Duarte, CA, USA*
Berley, Jordan - *AR DMRI, City of Hope, Duarte, CA, USA*
Hussey, Deborah - *AR DMRI, City of Hope, Duarte, CA, USA*
Kamali, Diba - *AR DMRI, City of Hope, Duarte, CA, USA*
Mishra, Pratibha - *AR DMRI, City of Hope, Duarte, CA, USA*
Guo, Chao - *Integrative Genomics Core-BRI, City of Hope, Duarte, CA, USA*
Kang, Tae-Hyuk - *Integrative Genomics Core-BRI, City of Hope, Duarte, CA, USA*
Wang, Jinhui - *Integrative Genomics Core-BRI, City of Hope, Duarte, CA, USA*
Wu, Xiwei - *Integrative Genomics Core-BRI, City of Hope, Duarte, CA, USA*
Riggs, Arthur - *AR DMRI, City of Hope, Duarte, CA, USA*
Tompkins, Joshua - *AR DMRI, City of Hope, Duarte, CA, USA*

Abstract: Epigenetics plays essential roles in the regulation of 3D structure of DNA, which is tightly connected with gene expression control. Dynamic changes of epigenetic marks, including DNA methylation (DNAme) affect cellular identity, therefore they are important for normal cell development and maturation. Loss of proper DNAme is noted in aging, cancer, diabetes, and several other human diseases. Our recent work has shown that integration of CpG-free single-stranded DNA (ssDNA) results in widespread CpG island (CGI) DNAme in human embryonic stem cells (hESCs), local to the integration point, which is maintained stably through differentiation into multiple lineages. Though taken from the inner cell mass, the epigenome of hESCs is said to be in a more “primed” state relative to mouse ESCs, and a more naive-like state can now be achieved by growing hESCs in specialized culture medium. Here, we report on global DNAme changes associated with naïve pluripotency and track the loss of engineered DNAme in this state. Remarkably, CGI Methylated Responses (CIMRs) are re-initiated when cells are cycled back to primed pluripotency, so long as the inducing CpG-free insert is retained. In conclusion, our data suggest that CpG-free insertion-based CIMR and the molecular machinery necessary for this event are restricted to primed pluripotency, and it indicates that we may be able to target the transition between naïve and primed pluripotency for future in vivo model development.

Keywords: Epigenetic engineering, DNA methylation, Primed pluripotency

779

MECHANISM OF EXOGENOUS RIBOSOME MEDIATED CELLULAR MULTIPOTENCY INDUCTION

Istiaq, Arif - *Brain Morphogenesis, Kumamoto University, Fukuoka, Japan*
Ohta, Kunimasa - *Stem Cell Biology, Kyushu University, Fukuoka, Japan*

Abstract: Ribosomes are well-known molecules that play a crucial role in translation by converting mRNA into proteins. However, we previously revealed that exogenous (exo) ribosomes can transdifferentiate human dermal fibroblast cells (HDFs) into three germ layer-derived cells (Ito et al.,2018). But, the underlying mechanism was unknown. When exo-ribosomes (purified from external origins like bacteria) are incorporated into the HDFs or Mouse embryonic fibroblasts (MEFs), they trigger a series of molecular events that lead to the suppression of cell lineage-specific genes (mesenchymal to epithelial transition) and the acquisition of stem-like properties expressing OCT4, Nanog, Sox2, KLF-4, Myc, and other essential stemness maintenance genes. The molecular events are characterized by nuclear localization of the exo-ribosome, cellular stress induction, and cell cycle arrest. Once exo-ribosomes enter a cell, they affect the chromatin landscape, influencing the open and closed chromatin regions in a way that favors stem-like characteristics in the incorporated cells. The transcriptome profile of the incorporated cells revealed that this gene switching process is gradual and distinct from traditional induced pluripotent stem cells (iPSCs). Comparing our process with iPSCs, our multipotency induction process is more natural, since ribosomes from live bacteria are used instead of artificially prepared viral vectors to overexpress genes like OCT4, Nanog, and Sox2. Our findings uncover a role for ribosomes in multipotency that has not been previously discovered. It also suggests that exo-ribosomes might be participating in the evolutionary process by interfering with cell fate determination.

Keywords: Exogenous Ribosomes, Induced Multipotency, Chromatin landscape

TOPIC: EYE AND RETINA

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MYCN INDUCES CELL-SPECIFIC TUMORIGENIC GROWTH IN RB1-PROFICIENT HUMAN RETINAL ORGANOID AND CHICKEN RETINA MODELS OF RETINOBLASTOMA

Zhang, Hanzhao - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Blixt, Maria - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Hellsand, Minas - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Konjusha, Dardan - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Stenfelt, Sonya - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Åkesson, Mikael - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Tararuk, Tatsiana - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Ring, Henrik - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*
Hallböök, Finn - *Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden*

Abstract: Retinoblastoma is a pediatric cancer originating in the neural retina. Although rare, retinoblastoma is the most common intraocular malignancy among children. It is most frequently caused by bi-allelic RB1 gene loss-of-function, while some secondary mutations can contribute to tumor progression. However, MYCN amplification has been found in some retinoblastoma cases and caused retinoblastoma independently of RB1 loss-of-function. Based on our previous research on chicken retinoblastoma model, we established RB1+/+ human embryonic stem cell-derived retinal organoids overexpressing MYCN with piggyBac transposon system. We used a 2D and 3D combination method to produce retinal organoids which formed layers of photoreceptors in later stage. MYCN overexpression was done by electroporation during Day39 to Day41 in culture. The piggyBac transposon system can transpose target genes to host genome in a "cut-and-paste" manner without footprint and the integration is permanent. In both chicken and human models, MYCN overexpression induced anaplastic growth in cells with markers for progenitors of photoreceptors and horizontal cells, not for amacrine and ganglion cells. MYCN overexpressing cells in retinal organoids still indicated photoreceptor/horizontal cell lineage even after 150 days in culture. This demonstrated that MYCN induced tumorigenic growth was cell type-specific. Moreover, majority of MYCN overexpressing cells in organoids showed markers for proliferation and mitosis. When comparing to RB1-/- retinal organoids, MYCN overexpressing cells in our model were positive for RXRγ but not for ARR3. This result showed that MYCN overexpressing RB1+/+ retinoblastoma model has a more undifferentiated and proliferating phenotype. In conclusion, our data demonstrate that MYCN overexpression is sufficient to initiate cell type-specific tumorigenic growth with proficient RB1. This model is at more undifferentiated status than RB1-/- retinoblastoma model. Our study presents a new in vivo retinoblastoma model with MYCN overexpression and proficient RB1, which can be considered as an important tool for carcinogenesis study and novel drug testing for retinoblastoma.

Keywords: MYCN, Retinoblastoma, Retinal organoid

TOPIC: NT - GENERAL

783

LOW-FREQUENCY REPETITIVE MAGNETIC STIMULATION SUPPRESSES NEUROBLASTOMA PROGRESSION BY DOWNREGULATING THE WNT/β-CATENIN SIGNALLING PATHWAY

Jo, Seongmoon - *Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*
Im, Sang Hee - *Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*
Baek, Dawoon - *Department of Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Seo, Dongryul - *Department of Medicine, Yonsei University College of Medicine, Seoul, Korea*
Ryu, Hayeon - *Department of Medicine, Yonsei University College of Medicine, Seoul, Korea*
Kim, Sung Hoon - *Department of Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Baek, Ahreum - *Department of Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Cho, Sung-Rae - *Department of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Neuroblastoma is a malignant tumour that mostly occurs in infants. The development of effective treatments is important to overcome the poor prognosis of high-risk neuroblastoma patients. Repetitive magnetic stimulation, which has been suggested as a potential therapy for various disorders, is a painless and non-invasive treatment. The purpose of the present study was to elucidate whether low-frequency repetitive magnetic stimulation (rMS) suppresses tumours in neuroblastoma models, and to explore the underlying mechanisms. In vitro and in vivo neuroblastoma models were treated with low-frequency rMS. To investigate potential mechanisms, RNA sequencing transcriptome analysis was performed. Quantitative real-time reverse transcription-polymerase chain reaction, western blotting, and immunohistochemistry were performed to validate the effects of low-frequency rMS treatment and unravel potential mechanisms. Low-frequency rMS treatment significantly suppressed cell proliferation and tumour progression in the models. Moreover, it was identified that the Wnt/β-catenin signalling pathway was downregulated by low-frequency rMS treatment. The Wnt/β-catenin signalling pathway activator, Wnt agonist, was found to counteract the effect of low-frequency rMS treatment on neuroblastoma models, while the Wnt/β-catenin signalling pathway inhibitor, Wnt antagonist, exhibited a tumour suppression effect on neuroblastoma models, similar to the effect of low-frequency rMS treatment. Taken together, our data demonstrated that low-frequency rMS treatment suppressed neuroblastoma progression by downregulating the Wnt/β-catenin signalling pathway, suggesting that low-frequency rMS treatment may be a potential therapeutic strategy for neuroblastoma.

Funding Source: This study was supported by the NRF-2020R1A2C1012019, KHIDI (HI21C1314), KFRM (21A0202L1 and 21C0715L1), and Hyundai Motor Chung Mong-Koo Foundation.
Keywords: Repetitive magnetic stimulation, Low-frequency, Neuroblastoma

TOPIC: EPITHELIAL SKIN

785

THE FATE TRACING OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED MELANOCYTE STEM CELLS

Zhou, Hang - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*
Liu, Li-Ping - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*
Wang, Yun - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*
Wang, Zi-Han - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*
Li, Yu-Mei - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*
Zheng, Yun-Wen - *Institute of Regenerative Medicine, and Affiliated Hospital of Jiangsu University, Jiangsu University, Zhenjiang, China*

Abstract: The existence of human melanocyte stem cells has been confirmed in recent studies, and its renewal, proliferation and differentiation potential may be the password for curation of the depigmentation disease. However, the human melanocyte stem cells have not been clearly defined since the identity included their cell surface markers remain unclear. It still fails to dynamically observe the cell differentiation process, also fails to realize the isolation and enrichment of melanocyte stem cells. Induced pluripotent stem cells (iPSCs) from homo species have the ability to differentiate into melanocytes, highly simulated the maturation process of melanocytes in human. Our established suspensive differentiation system highly mimicked the microenvironment of human fluid which simultaneously enhanced the efficiency of hiPSC differentiation into melanocytes. Based on the CRISPR/Cas9 gene editing, realized the co-expression of the visualizing protein and target genes. EGFP gene was integrated the into MITF gene, the key transcription factor that determines the melanocyte fate which maintains the proliferation and differentiation of melanocyte stem cells to melanocytes. During the differentiation of hiPSC into melanocytes, expression of MITF is observed in real time. The color change also indicates the maturation of melanocyte stem cells, the cloning formation capability and the cell differentiation were compared by the color rendering ratio. Except differentiated into mature melanocytes, MITF positive cells also have the potential to differentiate into other cells. Transplanting the MITF positive cells into mouse in situ, comparing with the unfractionated poll of hiPSC initiated melanocytes would indicate a renewable cell community, facilitated the characterization of human melanocyte stem cells. Our study creates a convenient in vitro dynamic observation model, which have a great potential in tracing the migration and differentiation of Melanocyte stem cells, breaks the obstacles in melanocyte stem cells traceability, provides a possibility for the surface marker definition in the near future, bring the dawn to depigmentation disorder patients.

Funding Source: This research was supported partly by the National Natural Science Foundation of China (82070638 and 82103766) and JSPS KAKENHI (JP18H02866).
Keywords: hiPSCs, Melanocyte stem cells, Visualization

POSTER SESSION I: ODD

6:30 PM – 7:30 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

201

EVALUATION OF ENHANCED IMMUNOMODULATORY PROPERTIES OF CANINE ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS VIA PRIMING WITH PRO-INFLAMMATORY CYTOKINES

Jo, Chan-Hee - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*
Choe, Yong-Ho - *Veterinary Obstetrics, College of Veterinary Medicine ,Gyeongsang National University, Jinju, Korea*
Kim, Saet-Byul - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*
Han, Jang-Ho - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*
Oh, Seong-Ju - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*
Rho, Gyu-Jin - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*
Lee, Sung-Lim - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju, Korea*

Abstract: Primed mesenchymal stem cells (MSCs) have revealed the remarkable potential to improve treatment efficiency for autoimmune diseases through their immunomodulatory properties. There is limited understanding of the cellular and immunomodulatory mechanisms of primed MSC with pro-inflammatory cytokines in canines. Therefore, we comparatively evaluated the variation of immunomodulatory factors according to combination types of pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-17) in canine adipose tissue-derived MSC (cAMSC). All procedures are authorized by the research ethics committee of GNU Animal Center (GNU-210329-M0033). 'Primed cAMSCs' were induced by three different conditions with pro-inflammatory cytokines for 48 hrs; single priming (IFN-γ, TNF-α, IL-17 alone), dual priming (IFN-γ+TNF-α, IFN-γ+ IL-17, and TNF-α+ IL-17), and triple priming (IFN-γ+TNF-α+IL-17). Immunomodulatory-related markers of all primed cAMSCs were analyzed at the mRNA level by qRT-PCR. The ability to suppress peripheral blood mononuclear cells (PBMC) proliferation with cAMSCs was assessed according to different combinations of pro-inflammatory cytokines. For PBMC analysis, PBMCs were isolated from blood of healthy dogs by Percoll density gradient centrifugation. After being activated by PHA-L, PBMCs were labeled with CFSE. The cells were then co-cultured for 96 hrs with primed cAMSCs. Priming of cAMSCs with IFN-γ upregulated the expression of IDO, COX2, PD-L1, and HGF at mRNA level. The expression level of IDO in cAMSCs primed with IFN-γ was significantly ($P < 0.001$) higher than cAMSCs primed with TNF-α and IL-17. According to PBMC analysis, IFN-γ primed cAMSCs predominantly reduced the proliferation of activated T-cells, whereas TNF-α and IL-17 primed cAMSCs have no effect. However, triple priming condition strongly enhanced the inhibitory effect of T-cell proliferation due to the additive effects of immunomodulatory soluble factors. In addition, COX2 expression is dramatically increased in triple priming combination rather than

single priming. In conclusion, although IFN-γ is recognized as a key activator of immunomodulatory factors, the combination of three major pro-inflammatory cytokines, IFN-γ, TNF-α, and IL-17 in cAMSCs is recommended to maximize the expression of IDO and COX2.

Funding Source: This study was supported by a grant from the National Research Foundation (NRF) of Korea, funded by the government of the Republic of Korea (grant #. NRF-2021R1A2C1007054)
Keywords: Canine adipose tissue-derived mesenchymal stem cells, immunomodulation, priming

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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DIFFERENTIATING IPSCS TO ENDOTHELIAL CELLS VIA ENDOGENOUS ACTIVATION OF TRANSCRIPTION FACTOR ETV2

Zhang, Qiao - *Biomedical Engineering, Duke University, Durham, NC, USA*
Yilmaz, Ceren - *Stem Cell Sciences, Hacettepe University, Ankara, Turkey*
Chen, Yicong - *Biomedical Engineering, Duke University, Durham, NC, USA*
Truskey, George - *Biomedical Engineering, Duke University, Durham, NC, USA*

Abstract: Tissue engineered blood vessels (TEBVs) made with iPSC-derived cells, which inherit the genetic background of the donor, present a promising platform to model vascular disease. Endothelial cells (ECs) form the intima of the blood vessels and their dysfunction initiates the development of many cardiovascular diseases, including atherosclerosis. However, current methods of EC differentiation suffer from low yield, long time to differentiate cells, and production of immature ECs. In this study, we whether endogenous activation of ETV2 (ETV2-ECs), a critical transcription factor in the development of endothelial cells and hematopoietic cells promoter more efficient EC differentiation than current methods. Guide RNA targeting the ETV2 upstream region of transcriptional start site was cloned into lentiviral backbone vector that expressed deactivated CRISPR Cas9 (dCas9) with regulatory elements (2xVP64), which activate gene transcription. iPSCs were transduced with CRISPR-dCAS9 2xVP64 ETV2 lentivirus at day 0 and after 7 days of differentiation, ECs were obtained by cell sorting for CD31/CD144. Stem cell genes OCT4, SOX2, NANOG, and UTF1 were expressed at high levels initially and decreased to low levels in the first 3 days. Mesoderm genes T and EOMES were expressed at low levels at the beginning and expressed at high levels transiently from day 2 to day4. Endothelial cell genes CD31, CD144, and vWF began expression at day 5 and expressed at a high level compared to WT cells. Most importantly, ETV2 had a high expression level transiently peaking on day 5 before the expression of EC genes. The temporal relationship of the gene expressions is consistent with the developmental process. Furthermore, these cells formed tubes in Matrigel and aligned to flow direction, which are signs of mature endothelial cells. Single-cell RNAseq and the initial data analysis showed similarity between ETV2-ECs and primary HUVEC cells in flow-mediated gene profiles of KLF2 and NRF2. Over 99.5% of ETV2-ECs were identified as 'Endothelial cells' with SingleR and Celldex analysis. In conclusion, in this study, we showed that mature ECs could be obtained from iPSCs in 7 days by endogenous activation of ETV2. This method provides a promising EC source for regenerative medicine.

Keywords: Tissue engineered blood vessels, ETV2 iPSC derived endothelial cells, CRISPRa of transcriptional factor

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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US STATE MEDICAL FREEDOM LAWS AND THEIR IMPACT ON UNPROVEN STEM CELL INTERVENTIONS

Matthews, Kirstin R. - *Baker Institute for Public Policy, Rice University, Houston, TX, USA*
Lowe, Samantha - *Baker Institute for Public Policy, Rice University, Houston, TX, USA*
Master, Zubin - *Center for Regenerative Medicine, Mayo Clinic, Rochester, MN, USA*

Abstract: Regulatory agencies in many countries are pushed to provide patients faster access to regenerative treatments. In the US, neoliberalism ideology promotes deregulation and limited government involvement for stem cell interventions (SCIs). While in many cases this is seen among national policies and politics, such efforts are also seen at the state level. In this presentation, we review different state bills filed from 2017 to present related to access to unproven SCIs. Enacted bills during this period fall into two broad categories based on intent: "Patient Awareness" and "Medical Freedom." The Patient Awareness bills require clinics advertisements to warn customers that the SCI being administered is unproven and has not been approved by the US Food and Drug Administration (FDA). The goal of these bills is to ensure patients seeking unproven SCIs understand the safety risks and limited science demonstrating efficacy. In contrast, Medical Freedom bills promote access to unproven SCI as an expansion of existing state Right to Try (RTT) laws and outline the conditions for which they can be given to patients. These laws also protect physicians who administer unproven SCI from reprimand by state medical boards. The first bill, passed in Texas in 2017, was used as model legislation and disseminated to other states by ALEC, a non-partisan organization for state legislators. We argue that Medical Freedom laws create significant social harms by undermining the regulatory authority of the FDA, confusing patients about the science and clinical readiness of SCIs, and fueling public distrust in the scientific and medical establishment. The stem cell scientific community has not paid sufficient attention to US state-based laws and should focus advocacy efforts towards stopping the passing or dismantling laws that promote the marketing of unproven SCIs.

Funding Source: KM & SL were supported by Rice University's International Stem Cell Policy Endowment. ZM was supported by a grant from the National Institute on Aging, NIH R21AG068620 & Mayo Clinic's Center for Regenerative Medicine.
Keywords: clinical translation, ethics and public policy, unproven stem cell interventions

TOPIC: HEMATOPOIETIC SYSTEM

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HEMATOPOIETIC STEM CELL TRANSPLANTATION PROMOTES FULLY MHC-MISMATCHED ISLET ALLOGRAFT TOLERANCE AND DIABETES REVERSAL

Bhagchandani, Preksha - *Developmental Biology, Stanford University, Stanford, CA, USA*
Chang, Charles - *Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA*
Poyser, Jessica - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Velasco, Brenda - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Zhao, Weichen - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Kwon, Hye-Sook - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Meyer, Everett - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Shizuru, Judith - *Medicine, Stanford University School of Medicine, Stanford, CA, USA*
Kim, Seung - *Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA*

Abstract: Organ transplantation strategies would benefit from development of reduced-intensity conditioning regimens to enable mixed hematopoietic chimerism and organ allotolerance. Mixed chimerism achieved by hematopoietic cell transplantation promotes tolerance of transplanted donor-matched solid organs and tissues, but currently requires toxic bone marrow conditioning, and entails risks of graft-versus-host disease (GVHD). We developed a chemotherapy-free, non-myeloablative conditioning regimen that achieves mixed chimerism and allograft tolerance across fully mismatched major histocompatibility complex (MHC) barriers. Durable multi-lineage mixed chimerism was achieved in immunocompetent mice using monoclonal antibody targeting of c-Kit, T-cell depleting antibodies, and low dose total body irradiation prior to transplantation of purified hematopoietic stem and progenitor cells. Mixed chimerism allowed for long-term tolerance of donor-matched pancreatic islet allografts in non-diabetic mice, without signs of GVHD. We applied this reduced-intensity conditioning protocol to diabetic B6 RIP-DTR mice that have a defined H2b MHC haplotype; a single dose of diphtheria toxin results in islet beta-cell ablation and rapid, fully penetrant diabetes in these immunocompetent mice. After diabetes induction and conditioning, fully MHC-mismatched donor-matched islets and hematopoietic cells were transplanted, resulting in 100% long-term correction of diabetes (n=9/9 mixed chimeric mice), with preservation of fertility and other measures of functional status, and without chronic immunosuppression or GVHD. Allotolerance is likely mediated by donor-derived thymic dendritic cells and host-derived peripheral regulatory T cells. We achieved similar long-term islet replacement and tolerance, and disease reversal in NOD mice with established autoimmune diabetes. These results provide a clinically translatable reduced-intensity conditioning regimen and cell transplantation protocol that achieves durable hematopoietic chimerism, promoting islet allograft tolerance and diabetes reversal. Our work also provides a paradigm for promoting hematopoietic chimerism to achieve transplantation allotolerance, and self-tolerance in autoimmunity.

Funding Source: Maternal & Child Health Research Institute (MCHRI), JDRF Northern California Center of Excellence, NIH (R01 DK107507; R01 DK108817; U01 DK123743; P30 DK116074), H.L. Snyder Foundation, Stanford Diabetes Research Center (SDRC).
Keywords: allotolerance, mixed chimerism, hematopoietic stem cell transplant

TOPIC: IMMUNE SYSTEM

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A NOVEL DEFINED ANIMAL COMPONENT FREE (ACF), PROTEIN FREE (PF), SALT BASE CRYOPRESERVATION SOLUTION, COMPOSED OF 5% DMSO DESIGNED FOR CELL-BASED THERAPIES.

Genser-Nir, Mira - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*
Daniliuc, Sharon - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*
Guri Ben Ari, Meital - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*
Goldstein, Nyra - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*
Teverovsky, Marina - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*
Fiorentini, David - *Research and Development, Biological Industries Sartorius, Kibbutz Beit Haemek, Israel*

Abstract: Cryopreservation is a crucial step for long term storage of cell-based products and for “off-the-shelf” cell therapy approaches. To date, the common practice is to use cryopreservation solutions composed of 10% DMSO or other toxic permeable cryoprotective agents (CPA), such as Ethylene Glycol. Exposure of cells to these CPAs can impact the quality, safety, and efficacy of the cellular product and clinical outcome. Facing strict regulatory requirements, the development of a defined cryopreservation solution with a reduced concentration of DMSO is required and holds a unique opportunity to advance the widespread implementation of cellular therapies. The current study presents the performance of NutriFreez D5, a novel defined ACF, PF, salt base cryopreservation solution with a reduced concentration of DMSO (5%). Post thaw cells viability, growth recovery and cell characterizations of human mesenchymal stem cells, pluripotent stem cells and immune cells, were evaluated after being frozen in NutriFreez D5. Continued work is invested in the development of the next generation, DMSO-free cryopreservation solution with non-toxic CPA alternatives.

Keywords: Cryopreservation solution, Mesenchymal Stem cells (hMSC), CELL-BASED THERAPIES

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QUERCETIN-HYDROGEL THREE DIMENSIONAL SCAFFOLD TO FACILITATE REGENERATION FOLLOWING SPINAL CORD INJURY

Ghoneim, Nehal I. - *Biotechnology, The American University in Cairo, 6th of October City, Giza, Egypt*
Ghoneim, Nehal - *Biotechnology Program, The American University in Cairo, Egypt*
Khalil, Islam - *Pharmaceutics, Misr University for Science and Technology, 6th of October, Egypt*
Magdy, Shireen - *Center of Excellence for Stem Cells and Regenerative Medicine (CESC), Zewail City of Science and Technology, 6th of October, Egypt*
Khalil, Eman - *Biology, The American University in Cairo, 6th of October, Egypt*
El-Mahdy, Manal - *Pathology, Ain Shams University, Cairo, Egypt*
El-Badri, Nagwa - *Center of Excellence for Stem cells and Regenerative Medicine (CESC), Zewail City of Science and Technology, 6th of October, Egypt*
Abdellatif, Ahmed - *Biology, The American University in Cairo, Egypt*

Abstract: Spinal cord injury (SCI) is a serious health issue that occurs with high incidence in young adults. As there is no gold standard treatment for spinal cord injury, new therapeutic strategies with neuro-regenerative and neuro-protective prosperities are required. Recently several studies have shown that the combination of cellular therapy with Gelatin methacryloyl (GelMA) hydrogel biomaterials in spinal cord injury could provide a potential solution for regeneration of spinal cord. Further improvement is required to decrease the inflammatory process at the site of the injury to promote the regeneration. Quercetin nanoparticles could provide a synergistic effect when combined with the ASCs laden 3D GelMA hydrogels to support the regeneration in SCI rat model. In this study, quercetin nanoparticles were loaded into the three-dimensional (3D) soft gelatin methacryloyl (GelMA) (5%) scaffold supplemented with adipose tissue-derived stromal cells (ASCs) and then they were implanted into the hemisection site of the rat spinal cord. We have found that ASCs that were photoencapsulated in the 3D GelMA hydrogel loaded with 10 µmol quercetin nanoparticles demonstrated higher proliferation rate in vitro after 7 days. Interestingly, the 3D GelMA hydrogel loaded with quercetin showed less angiogenic property than the hydrogel without quercetin when administered to E-7.5 days chick embryos that supports its anti-inflammatory and antioxidant effect. Besides, quercetin nanoparticles have improved the mechanical properties of the hydrogel scaffold to support the regeneration of the spinal cord. Also, our results showed that when ASCs laden quercetin-hydrogels were implanted into the hemisection site of the rat spinal cord, the hydrogel filled the destructed gap and organized tissue were noted at the site of injury. Therefore, the ASCs laden quercetin-hydrogels is a promising therapeutic strategy to trigger functional regeneration of the spinal cord.

Funding Source: This work is funded by graduate research support grant from The American University of Cairo .
Keywords: Quercetin, 3D hydrogel, Spinal cord injury (SCI)

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IDENTIFICATION OF A NOVEL CELL SURFACE MARKER WITH IMPROVED SPECIFICITY TOWARDS VENTRAL MIDBRAIN DOPAMINERGIC PROGENITOR CELLS

Schörling, Alrik - *Department of Experimental Medical Science, Lund University, Lund, Sweden*
Rifes, Pedro - *Department of Neuroscience, University of Copenhagen, Denmark*
Singh Rathore, Gaurav - *Department of Neuroscience, University of Copenhagen, Denmark*
Salvador, Alison - *Department of Neuroscience, Lund University, Lund, Sweden*
Holm, Amalie - *Department of Neuroscience, University of Copenhagen, Denmark*
Parmar, Malin - *Department of Experimental Medical Science, Lund University, Lund, Sweden*
Zhang, Yu - *Cell Therapy Development and Manufacturing, Takara Bio Inc., Göteborg, Sweden*
Kirkeby, Agnete - *Department of Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Human pluripotent stem cells are a promising source of caudal ventral midbrain (cVM) progenitor cells that are used in cell replacement therapy for Parkinson's disease. However, heterogeneity in cell composition is inevitable in the cell differentiations. Cell surface markers predictive of functional maturation of cVM progenitors to ventral midbrain dopaminergic (vmDA) neurons have potential as a convenient quality control and to enrich for the target cell type. By performing single-cell RNA sequencing of our in vitro model of the developing neuronal tube, we identified a candidate cell surface marker of cVM progenitors. Benchmarking the specificity of this marker to cVM neural progenitor populations compared previously published cVM progenitor markers by using flow cytometry combined with in situ modeling revealed that the previously published surface marker trophoblast glycoprotein (TPBG) as well as our newly identified marker are the most enriched for cVM progenitors. However, our novel marker showed a higher reproducibility than TPBG, ergo is more suitable as a flow cytometry-based quality control. The marker correlated to genes indicative of a vmDA fate. Cells positive for the marker, sorted from a mix of neuronal progenitor cells, yielded behavioural recovery in a Parkinsonian rat model, but cells negative for the marker did not and had lower innervation than cells positive for the marker. Taken together, our new cVM cell surface marker outperforms previously published markers, and will be useful in upcoming clinical trials exploring cell therapy as Parkinson's disease treatment.

Funding Source: Wallenberg Centre for Molecular Medicine
Keywords: Cell surface marker, Ventral midbrain dopaminergic neurons, Human pluripotent stem cells

TOPIC: PANCREAS

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GENETIC ENGINEERING OF STEM CELL DERIVED PANCREATIC BETA-LIKE CELLS CONFERS PROTECTION FROM AUTOIMMUNE DIABETES

Castro-Gutierrez, Roberto - *Barbara Davis Center/ Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Santini-Gonzalez, Jorge - *Bioengineering, University of Florida, Gainesville, FL, USA*
Alkanani, Aimon - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
McDaniel, Kristen - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Anderson, Amanda - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Nakayama, Maki - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Michels, Aaron - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Phelps, Edward - *Bioengineering, University of Florida, Gainesville, FL, USA*
Russ, Holger - *Barbara Davis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: Type 1 diabetes (T1D) results from an autoimmune destruction of pancreatic beta cells mediated by diabetogenic CD8+ T cells. Transplantation of cadaveric beta cells into patients with T1D has been proposed as a cure but has been hampered by donor shortage. We and others have described the generation of an abundant source of functional stem cell derived beta-like cells (sBC) that can rescue diabetes after transplantation into preclinical animal models. However, recurring autoimmunity is a remaining hurdle to overcome. Our goal is to model and revert human autoimmune diabetes by protecting sBC from (auto-)immune destruction. To test sBC immunogenicity against diabetogenic CD8+ T cells we generated a novel in vitro co-culture assay system. sBC derived from human pluripotent stem cells (hPSC) from donors with and without T1D are co-cultured with diabetogenic CD8+ T cells. Our data shows that diabetogenic CD8+ T cells can directly interact and destroy sBC derived from multiple hPSC lines. To avoid this immune destruction, we employed genetic engineering to delete human leukocyte antigen (HLA) class I molecules and overexpress the PD-L1 receptor on sBC. These modifications result in complete protection from diabetogenic CD8+ T cell recognition in vitro. Furthermore, to validate this protection in vivo, we transplanted genetically engineered sBC into a novel humanized HLA-matched autoimmune diabetic mouse model. sBC transplantation under the kidney capsule of these mice resulted in CD3+ T cell infiltration indicative of a functional adaptive immune responds. Moreover, preliminary results show that sBC overexpressing PD-L1 exhibit greater survival compared to controls, suggesting efficacy of our immune modulatory approach in vivo. Here, we present novel in vitro and in vivo platforms to interrogate the immune-beta cell interactions in a human autoimmune context by (i) co-culture of sBC with human diabetogenic CD8+ T cells and (ii) a humanized HLA-matched diabetic mouse model. Furthermore, we demonstrate that manipulation of HLA and PD-L1 receptors provide sBC protection from an autoimmune diabetic immune attack. Taken together, we postulate genetically engineered stem cell derived beta cells as a cell therapy for patients suffering from T1D.

Keywords: Autoimmune diabetes, cell therapy, beta cell replacement

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ENRICHMENT OF STEM CELL DERIVED PANCREATIC BETA-LIKE CELLS AND CONTROLLED GRAFT SIZE THROUGH PHARMACOLOGICAL REMOVAL OF PROLIFERATING CELLS

Shilleh, Ali H. - *Department of Pediatrics, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA*
Beard, Scott - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Russ, Holger - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*

Abstract: Transplantation of purified human cadaveric islets into type one diabetic (T1D) patients results in ~35 months of insulin independence, tremendously improving quality of life. As such, cell replacement therapy has been proposed as a potential cure, however, the lack of an abundant source of donor beta cells has severely hampered widespread application. We and others have recently developed a step wise differentiation protocol for large-scale production of stem cell derived insulin producing beta-like cells (sBC). In vivo studies demonstrate sBC graft ability to reverse diabetes in animal models. However, uncontrolled graft growth has been a concern and multiple groups reported the presences of unwanted proliferative cystic structures within sBC grafts. Current direct differentiation protocols do not generate pure sBC but consist of only approximately 30% insulin expressing cells with many additional off target cell types some of which are proliferative. Thus, approaches to enrich for sBC and/or ablate unwanted cell types are needed. Here we show the selective ablation of proliferative cell population marked by SOX9 expression within sBC clusters by simple pharmacological treatment in vitro. qPCR, immunofluorescence and flow cytometry analysis showed that this treatment concomitantly enriches for insulin producing sBC by 1.5x fold independent of initial differentiation efficiency. Treated enriched sBC clusters show improved function as assayed by Ca2+ imaging and dynamic glucose stimulated insulin secretion perfusion analysis in vitro. In vivo transplantation of treated sBC clusters controls graft size, with the absence of unwanted proliferative cystic structures and 4x and 10x fold decrease in SOX9 and KI67 expressing cells, respectively. Overall, our study provides novel insights how to minimize tumor formation in cell therapy approaches and refine the use of sBC for the treatment T1D patients.

Keywords: Direct differentiation, pancreatic beta cell, Chemotherapy drugs, teratoma prevention, Human pluripotent stem cells, cell therapy

TOPIC: PLURIPOTENT STEM CELLS

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AUTOMATED BIOREACTOR SYSTEM TO SCALE UP PRODUCTION OF HUMAN INDUCED PLURIPOTENT STEM CELL EMBRYOID BODIES FOR BIOPRINTING

Ho, Debbie L.L. - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Devine, Sean - *Sartorius Stedim, Sartorius Stedim North America, Inc., Bohemia, NY, USA*
Du, Jianyi - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Hamfeldt, Art - *Data Analytics, Sartorius Stedim North America, Inc., Bohemia, NY, USA*
He, Mengdi - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Katikireddy, Kishore - *Sartorius Stedim, Sartorius Stedim North America, Inc., Bohemia, NY, USA*
Klinger, Danielle - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Lee, Stacey - *Bioengineering, Stanford University, Stanford, CA, USA*
Leng, Hope - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Mitchell, Marc - *Sartorius Stedim, Sartorius Stedim North America, Inc., Bohemia, NY, USA*
Sinha, Soham - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Skylar-Scott, Mark - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Tam, Tony - *Bioengineering, Stanford University, Palo Alto, CA, USA*
Traul, Donald - *Sartorius Stedim, Sartorius Stedim North America, Inc., Bohemia, NY, USA*
Vicard, Quentin - *Sartorius Stedim, Sartorius Stedim France S.A.S., Aubagne Cedex, France*
Weiss, Jonathan - *Bioengineering, Stanford University, Palo Alto, CA, USA*

Abstract: Billions of cells are needed for tissue engineering and bioprinting organs for transplantation. Traditional 2D cultures are not inherently scalable to continuously culture billions of cells, which require large surface area and handling. Here, we demonstrate the formation and continuous culture of human induced pluripotent stem cell (hiPSC) embryoid bodies (EBs) using an automated 250 mL stirred tank bioreactor system (Ambr250 Modular, Sartorius). In cultures over five days, we characterized the impact of seeding density, initial and final impeller rotation speeds, on the daily trends of viable cell density, fold-expansion, size and circularity of the hiPSC EBs. Furthermore, iterations of computational multivariate analyses and wet-lab experiments were conducted to refine the ideal culture conditions such as seeding density, initial and final impeller speeds, gas input and pH controls. In the optimal culture condition, our approach achieved a 19-fold expansion of cells at a density of 4.2 million cells/mL, while maintaining > 94% expression of pluripotency markers Oct4, Nanog, SSEA-4, and TRA-1-60. Furthermore, we tuned the impeller speed to obtain EBs with desired diameters ranging from 250 - 300 µm for downstream organoid differentiation or 300 - 450 µm for maintenance and continuous culture. Using this system, we generated 1 billion cells per vessel which surpasses densities achieved by conventional 2D cultures per volume of media. Cell density, EB morphology, and pluripotency marker expression were maintained after three serial passages. These EBs differentiated into derivatives of the three germ layers, including cardiac, vascular, cortical and intestinal organoids. Finally, the EB cultures were compacted into a cellularly-dense bioink for rheological characterization and 3D bioprinting. The printed structures were successfully differentiated into ectodermal and mesodermal tissue. This work provides avenues for our next efforts to scale culture volumes to the 10 L scale to produce billions of hiPSC-derived cells for bioprinting applications.

Funding Source: Additional Ventures Innovation Fund, Additional Ventures Cures Collaborative, Sartorius Stedim
Keywords: Bioreactor, Scale-up, human induced pluripotent stem cells

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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PATIENT AND PUBLIC INVOLVEMENT/ ENGAGEMENT (PPI/E) AND BENEFIT SHARING IN STEM CELL RESEARCH: A SURVEY OF PATIENTS IN JAPAN

Watanabe, Saori - *Department of Public Policy - Institute of Medical Science, The University of Tokyo, Minato-city, Japan*
Muto, Kaori - *Department of Public Policy, Institute of Medical Science, University of Tokyo, Minato-ku, Japan*
Yui, Hideki - *Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan*
Yamagata, Zentaro - *Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan*
Yashiro, Yoshimi - *Integrated Research Initiative for Living Well with Dementia, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Itabashi-ku, Japan*

Abstract: With the practical application of regenerative medicine and stem cell research (SCR) as a significant part of therapy development, patients' active involvement in SCR may contribute to smoother conduct of research, including reduced participant risks, improved enrollment, and continuity of cell donors and information. Additionally, as SCR enters the phase of commercialization and policies are being made to encourage the participation of industry and private companies, discussions regarding benefit sharing in SCR have not yet been sufficiently conducted. Based on the analysis of an awareness survey of patients, this research aimed to identify issues of patient involvement and benefit sharing in SCR in Japan. In the survey, the "patients" were defined as adult respondents registered with the survey company; they were aged 20 years or older and had visited a hospital or had a history of any disease in the past year. The patients' responses to the questionnaire were anonymous and were collected online from December 23 to 27, 2021. The number of valid responses was N = 4,465. When asked about the support required for patients and families to promote PPI/E, educational factors were frequently selected, such as providing educational opportunities for patients and families (44.5%). Respondents also selected financial support for patient associations (36.3%) and reimbursement for individuals (21.4%). In the questionnaire on benefit sharing, 60.9% of the respondents answered that they would approve of companies selling products made from patient-derived iPSCs; however,

only 7.9% strongly approved it on a four-point scale, indicating that most respondents were reserved in their attitudes. As for the return of profits made by companies, 54.7% favored returning profits to the patient community as a whole, such as patient associations, and 35.3% favored returning profits to individuals who donated cells or data. Only 10% responded that companies do not need to return profits to the society. The analysis suggests classic but unresolved ethical and societal issues for responsible SCR, such as developing a PPI/E educational program suitable for SCR and the need for continuous dialogue with companies regarding social justice toward giving back to the community via benefit sharing.

Funding Source: This research was supported by AMED under Grant Number JP21bm0904002.
Keywords: Patient and Public Involvement/Engagement (PPI/E), Benefit Sharing, Ethics and Public Policy

TOPIC: IMMUNE SYSTEM

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OFF-THE-SHELF IPSC-DERIVED NATURE KILLER CELLS WITH ENHANCED EXPRESSION OF NK ACTIVATING RECEPTORS FOR THE TREATMENT OF SOLID TUMORS

Yang, Luhan - *Qihan Biotech, Qihan Biotech, Hangzhou, China*
He, Xiangjun - *ERD, Qihan Biotech, Hangzhou, China*
Wang, Yiyun - *The First Affiliated Hospital, Zhejiang University, Hangzhou, China*
Wang, Linqin - *The First Affiliated Hospital, Zhejiang University, Hangzhou, China*
Shao, Mi - *The First Affiliated Hospital, Zhejiang University, Hangzhou, China*
Le, Lei - *ERD, Qihan Biotech, Hangzhou, China*
Yue, Yanan - *Research and Development, Qihan Biotech, Hangzhou, China*
Gao, Yangbin - *Research and Development, Qihan Biotech, Hangzhou, China*
George, Church - *Department of Genetics, Harvard University, Boston, MA, USA*
Huang, He - *The First Affiliated Hospital, Zhejiang University, Hangzhou, China*

Abstract: CAR-T cells have shown clinical success for treating hematologic malignancies. However, the efficacy in treating solid tumors has been limited, mainly due to tumor heterogeneity and immunosuppressive tumor microenvironment (TME). Natural Killer (NK) cells derived from engineered human iPSCs hold great potential to become the next-generation allogeneic cell therapy products. NK cells exert anti-tumor activity through the net outcome of stimulatory and repressive signals from various activating and inhibitory receptors. Some of the ligands for NK cell activation are highly expressed on many solid tumors. Thus, overexpression of activating receptors on NK cells might further boost their killing capacity towards those tumor cells. Here we report QN-030 ("Super NK cells"), an investigational, iPSC-derived NK cell product with enhanced expression of a variety of activating receptors (NKG2D and NCRs) intended for the treatment of solid tumors. QN-030 is generated from an allogeneic iPSC clone engineered with three anti-tumor modalities, including a constitutively expressed activating receptor to boost NK killing capacity, a high-affinity, non-cleavable CD16 (hnCD16) to enhance antibody-dependent cell-mediated cytotoxicity (ADCC), and an IL-15 molecule to increase the persistence of allogeneic cells in patients. In this study, we first engineered a small library of human iPSCs, each one carrying 1-3 of engineered NK activating receptors on top of hnCD16 and IL-15. We then differentiated the engineered iPSCs into NK cells and conducted both in vitro and in vivo screening by using a variety of solid tumor cell lines. We identified QN-030 as the lead, which demonstrated superior anti-tumor activity among the candidates both in vitro and in mouse PDX model. QN-030 cells can be mass-produced in a cGMP process, have phenotype comparable to healthy donor NK cells, and are functionally potent against multiple solid tumor models. When administered in combination with monoclonal antibody, QN-030 demonstrated superior ADCC to unmodified iPSC-derived NK cells in mouse PDX models. Close monitoring of NKG2D/NCR expression revealed the potential mechanism of enhanced tumor killing activity. Together, we have engineered iPSC-derived QN-030 NK cells as a promising clinical drug candidate for treatment of solid tumors.

Keywords: Natural killer cells, iPSC, Solid tumor

TOPIC: MUSCULOSKELETAL

719

THE CLINICAL AND RADIOLOGICAL EFFECTIVENESS OF AUTOLOGOUS BONE MARROW DERIVED OSTEOBLASTS (ABMDO) IN THE MANAGEMENT OF AVASCULAR NECROSIS OF THE FEMORAL HEAD (ANFH) IN SICKLE CELL DISEASE (SCD)

Sadat-Ali, Mir - *Orthopedic Surgery, College of Medicine, Imam AbdulRahman AlFaisal University, Dammam, Alkhobar, Saudi Arabia*
Acharya, Sadananda - *Stem Cell Unit, Imam AbdulRahamn Bin Faisal University, Dammam, Dammam, Saudi Arabia*
Al Muhaish, Mona - *Radiology, Imam AbdulRahman Bin Faisal University, Dammam, AlHobar, Saudi Arabia*
AlOmran, Abdallah - *Orthopedic Surgery, Imam AbdulRahman Bin Faisal Un iversity, AlKhobar, Saudi Arabia*
AlTabash, Khalid - *Orthopedic Surgery, Imam AbdulRahman Bin Faisal University, Dammam, AlKhobar, Saudi Arabia*
Hegazi, Tarek - *Radiology, Imam AbdulRahman Bin Faisal University, Dammam, AlKhoabr, Saudi Arabia*

Abstract: Avascular necrosis of femoral head is a common Issue faced by orthopaedic surgeons which ranges between 10-18% but in sickle cell Disease the incidence reaches 30%. We report here our experience with injection of ABMDO to manage ANFH and report our long term results, progression of the ANFH if any and delay in THA (Total Hip Arthoplasty). Sixty-Three (63) consecutive patients with SCD with ANFH were examined, thoroughly investigated and those who had ANFH < grade II were consented to receive ABMDO. Pre-operatively patients were clinically assessed using Visual analogue scale (VAS), Modified Harris Hips Score (MHHS). Ten milliliter of bone marrow was aspirated under local anesthesia and was placed in 20 CC culture media. Osteoblasts were cultured from the bone marrow aspirated. Under anesthesia using 3 mm cannulated drill, the osteonecrosed lesion was drilled and 5 million osteoblasts were injected at the lesion site. Patients were evaluated in out patient clinic after two weeks. At four months a repeat MRI was done and patients were followed up a minimum for 2 years. The average age was 25.93±5.48 years. There were 41 (65%) females and 22 (35%) males. The

mean hemoglobin S was 83.2±5.1 percent. The average follow up was 49.05±12.9 (range 24-60) months. VAS significantly improved from 7.79±1.06 at 2 weeks 4.07±1.08 p< 0.0001 continued to improve for the next 24 months 2.38±0.55 (P< 0.0001). MHHS improved from 41.77±5.37 to 73.19± 6.48 at 4 months (P< 0.001) and at 24 months it was 88.93±3.6 (p< 0.001). A comparison of the MRI's of before and after osteoblast implantation revealed new bone formation and amelioration of the avascular lesions. Three patients were unhappy with the outcome and one patients repeated attacks of the vaso-occlusive crisis within six months of the osteoblasts injection. The results give credence to our earlier short follow up results that osteoblasts transplantation has a great potential in healing of avascular lesions. Our study fits the criteria of Phase II clinical trial and We believe a larger study equivalent to Phase III numbers and include patients not only with sickle cell disease but also steroid induced and idiopathic avascular necrosis.

Funding Source: None
Keywords: Stem cell, Osteoblasts, Avascular Necrosis of Femoral Head

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

721

SAFETY OF COMPLEX STEM CELL PRODUCTS APPLICATIONS: NOVELTY OF THE KNOWN

Petriv, Taras - *QR Health Solutions, Department of Restorative Neurosurgery, QR Health Solutions, LLC "Hemafund", Romodanov Neurosurgery Institute of NAMSU, Kiev, Ukraine*
Isaev, Sergii - *Main Department, QR Health Solutions, Kyiv, Ukraine*
Leshkovych, Karina - *Main Department, QR Health Solutions, Kyiv, Ukraine*
Skuratov, Alexandr - *Department for Traumatology and Orthopedics of Children, SI "The Institute of Traumatology and Orthopedics" by NAMS of Ukraine, Kyiv, Ukraine*
Tatarchuk, Michael - *Department of Restorative Neurosurgery, SI "Romodanov Institute of Neurosurgery of National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine*
Tsymbaliuk, Yaroslav - *Department of Restorative Neurosurgery, SI"Romodanov Institute of Neurosurgery of National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine*
Tsymbaliuk, Yulia - *Department of Restorative Neurosurgery, SI"Romodanov Institute of Neurosurgery of National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine*
Tsymbaliuk, Vitaliy - *Head of NAMSU, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine*

Abstract: Umbilical cord mesenchymal stem cells (UC-MSC) and umbilical cord blood stem cells (UCB-SC) successfully used in the treatment of a wide range of diseases. Despite the rapid development of advanced medical treatment, complex solutions are needed to increase the effectiveness of treatment. There is a synergistic interaction of different types of stem cells at different levels. The use of complex cellular products based on several types of stem cells, can significantly increase the effectiveness of their use. Objective of the study was to investigate the safety of complex UC-MSC+UCB-SC products applications. Patients were examined according to the clinical research protocol approved by the Academic Council of the National Academy of Medical Sciences of Ukraine. The study involved 10 patients: 2 patients with autistic spectrum disorders (ASD), 5 patients with cerebral palsy (CP), 1 patient with rheumatoid arthritis (RA), 2 patients with diabetes type 1. The mean age of patients was 3.21±0,39 years. Patients underwent intravenous application of US-MCS+UCB-SC complex product (allogenic MSC which met the phenotypic and morphologic criteria of the MCS and UCB-SC from autologous samples) once. The clinical condition of patients was assessed after 2 hours, 6 hours, and 24 hours. Repeated clinical examination was performed after 6 months. No serious adverse reactions were noted. In 2 patients there was a headache of medium intensity for 6-8 hours. No increase in body temperature was recorded in any of the patients. the first nonspecific changes in the form of improvement of mental activity, tendencies to normalization of level of glucose in blood and expansion of motor activity were observed at patients after first week after treatment. The use of a complex cellular product is safe and effective. The synergistic effect of its use leads to a faster clinical effect. Weakness of the study are the small number of patients, the lack of a comparison group and different kind of conditions. Application of US-MCS+UCB-SC complex product is promising, but it is necessary to continue research in this area.

Keywords: Complex stem cell product, perinatal stem cells, safety of clinical application

POSTER SESSION I: ODD

6:30 PM – 7:30 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

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USING A CRISPR/CAS9 IN VITRO HUMAN INDUCED PLURIPOTENT STEM CELL MODEL TO INVESTIGATE THE ROLE OF PERLECAN IN CARDIOVASCULAR FIBROTIC DISEASE

Johnson, Benjamin B. - *MED, University of East Anglia, Norwich, UK*
Holmes, Terri - *Med, University of East Anglia, Norwich, UK*
Merry, Cathy - *Biological Science, University of Nottingham, UK*
Denning, Chris - *Bio, University of Nottingham, UK*
Whitelock, John - *Bio, UNSW, Sydney, Australia*
Smith, James - *Med, University of East Anglia, Norwich, UK*

Abstract: Cardiac fibrosis and heart remodelling is initially essential for allowing continued cardiac function following myocardial infarction (MI). However, a prolonged inflammatory response can progress a patient towards heart failure. The key cell types in this process include cardiac fibroblasts (CFs), myofibroblasts (MFs) and cardiomyocytes (CMs). Heparan sulfate proteoglycans (HSPGs) are an area of interest in cardiac healing and wound repair, with one such HSPG being perlecan (HSPG2). Perlecan is a large basement membrane protein known for the binding of growth factors and extracellular matrix, key factors in cardiac fibrosis, as well as being essential for the innate function of the heart. Human induced pluripotent stem cells (hiPSCs) offer a powerful tool in modelling the key cell types and interactions during cardiac fibrosis. A perlecan deficient iPSC line was created in Nottingham by Dr James Smith using CRISPR/Cas9. This line was used to produce key cells types involved in cardiac fibrosis through differentiation, followed by further functional analysis, to

model the cell specific role of Perlecan. Perlecan deficient iPSCs showed a 58% reduction in perlecan expression using qRT-PCR, confirming a heterozygous knockdown. hiPSCs were successfully able to differentiate to CFs, MFs, and CMs. The CFs with attenuated perlecan showed reduced proliferation, metabolic output, and ECM secretion. Additionally, the CMs with attenuated perlecan displayed altered glycolysis metabolism. Our results show how CF and CM function is altered by reduced perlecan expression. Proliferation of CFs is essential in response to cardiac injury, suggesting a role for perlecan in the healthy response. Increased foetal-like metabolism in perlecan deficient CMs suggested a role of perlecan in the maturation of hiPSC derived CMs. Co-culture systems showed a high density of perlecan ECM secreted in the formation of cardiac scarring; this was lacking in perlecan deficient CFs.

Keywords: Cardiac Disease Model, CRISPR/Cas9, Proteoglycans

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PRECLINICAL CRISPR TREATMENTS TO CORRECT DUCHENNE MUSCULAR DYSTROPHY IN PATIENT-SPECIFIC CARDIOMYOCYTES AND TISSUES

Gutiérrez-Gutiérrez, Óscar - *Stem Cell Unit, Clinic for Cardiology and Pneumology University Medical Center Göttingen, Göttingen, Germany, University Medical Center, Göttingen, Germany*
Richter, Otilie - *Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Germany*
Tiburcy, Malte - *Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany*
Shahriyari, Mina - *Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany*
Schoger, Eric - *Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany*
Zimmermann, Wolfram-Hubertus - *Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany*
Cyganek, Lukas - *Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Germany*

Abstract: Duchenne muscular dystrophy (DMD) is a rare disease characterized by progressive muscle weakness and muscle loss, which leads to respiratory disabilities and cardiomyopathies during adulthood and ultimately death. This X-linked recessive disorder has an incidence of 1 in 3,500 males and is caused by mutations in the dystrophin gene. Dystrophin is a giant protein expressed mainly in muscle cells whose function is to stabilize the muscle fibers by connecting the cytoskeleton to the extracellular matrix. It has been reported more than 5,000 different DMD mutations, mostly out-of-frame deletions of one or more exons, which give rise to frameshift, premature termination of translation and lack of dystrophin. Interestingly, in-frame deletions resulting in truncated functional dystrophins can give rise to milder muscular dystrophies or even asymptomatic clinical phenotypes. In our study, we aimed to develop personalized CRISPR/Cas9-based gene therapy strategies in several DMD patients, converting out-of-frame deletions in in-frame deletions by exon skipping. To achieve this, patient-specific induced pluripotent stem cells (iPSCs) were generated, differentiated into cardiac muscle cells, and phenotyped at the cellular and tissue level. Patients' iPSC-derived cardiomyocytes (iPSC-CMs) confirmed disrupted reading frame, lack of dystrophin protein and severely reduced contractile performance. In order to induce exon skipping and reframing of the coding sequence, different single guide or dual-guide CRISPR/Cas9 approaches targeting the adjacent exons were tested. Thus, corrected iPSC-CMs displayed efficient exon skipping and restoration of dystrophin expression. Excitingly, engineered heart muscle generated from corrected cardiomyocytes presented a restoration of the contractile function. Finally, gene correction in cardiomyocytes was achieved by the use of all-in-one AAV vectors containing smaller Cas9 variants and the guide RNA cassettes in a single construct. Altogether, patient-specific iPSC-CMs, engineered heart muscle, and all-in-one AAV vectors, represent an excellent platform to test different clinically translatable CRISPR/Cas9-based exon skipping strategies for treating DMD.

Keywords: Duchenne Muscular Dystrophy, CRISPR/Cas9, Exon skipping

307

MILLIPILLAR: A PLATFORM FOR THE GENERATION AND REAL-TIME ASSESSMENT OF ENGINEERED HUMAN CARDIAC TISSUES FOR PATIENT-SPECIFIC DISEASE MODELING

Nash, Trevor R. - *Biomedical Engineering, Columbia University, New York, NY, USA*
Fleischer, Sharon - *Biomedical Engineering, Columbia University, New York, NY, USA*
Tamargo, Manuel - *Biomedical Engineering, Columbia University, New York, NY, USA*
Kim, Youngbin - *Biomedical Engineering, Columbia University, New York, NY, USA*
Vila, Olaia - *Biomedical Engineering, Columbia University, New York, NY, USA*
Yeager, Keith - *Biomedical Engineering, Columbia University, New York, NY, USA*
Summers, Max - *Biomedical Engineering, Columbia University, New York, NY, USA*
Lock, Roberta - *Biomedical Engineering, Columbia University, New York, NY, USA*
Zhao, Yimu - *Biomedical Engineering, Columbia University, New York, NY, USA*
Liberman, Martin - *Biomedical Engineering, Columbia University, New York, NY, USA*
Chavez, Miguel - *Biomedical Engineering, Columbia University, New York, NY, USA*
Vunjak-Novakovic, Gordana - *Biomedical Engineering, Columbia University, New York, NY, USA*

Abstract: Engineered cardiac tissues starting from induced pluripotent stem cells (iPSCs) are becoming increasingly powerful tools for drug discovery, pharmacology, and modeling cardiovascular development and disease. Although numerous engineered cardiac tissue platforms exist, most require specialized equipment and expertise that are only accessible to a limited number of academic labs, if any, beyond that of the group that developed the platform. As a result, few labs have been able to harness this powerful technology for their own applications and to study their own questions. And even among this limited group, the reproducibility of methodologies and tissue features has been challenging. To address these issues, we developed a facile technology (known as "milliPillar") that covers the entire pipeline required for studies of engineered cardiac tissues. It includes a bioreactor platform with flexible pillars to support the tissues and provide electrical stimulation of each tissue that has been optimized for consistent fabrication of tissues at high yield. We describe methodologies for (i) platform fabrication, (ii) tissue generation, (iii) electrical stimulation, (iv) automated real-time data acquisition, and (v) ad-

vanced video analyses. These methodologies enable objective automated analysis of tissue contractility, force generation, and calcium dynamics. We validated this platform and demonstrated its versatility by fabricating tissues using multiple hydrogel compositions and with cardiomyocytes derived from multiple iPSC lines in combination with different types of stromal cell populations (i.e. primary and iPSC-derived cardiac fibroblasts). We also validated the long-term culture of tissues within the platform (>3 months) and demonstrated the ability to enhance functional maturity of tissues by electromechanical stimulation during extended culture times. The platform is currently used to study a variety of cardiovascular diseases including ischemia reperfusion injury, autoimmune-mediated myocarditis, restrictive cardiomyopathy, drug-induced cardiotoxicity, and exposure to radiation.

Keywords: Engineered Cardiac Tissue, iPSC-Cardiomyocytes, Bioreactor

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HEAD-TO-HEAD COMPARISON OF HIPSC-DERIVED CARDIAC MODELS IN 2D, 2D ALIGNED, MICROTISSUE AND ENGINEERED HEART TISSUE MODELS AGAINST A PANEL OF BENCHMARK CARDIAC DRUGS

Dias Brescia, Marcella - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*
Brescia, M. - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*
Casti, P. - *Electronic Engineering, University of Rome Tor Vergata, Rome, Italy*
Sala, L. - *Center for Cardiac Arrhythmias of Genetic Origin, Instituto Auxologico Italiano, Milan, Italy*
Dostanić, M. - *Department of Microeletronics, TU Delft, Netherlands*
Mol, M.P.H. - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*
Wiendels, M. - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*
Mencattini, A. - *Electronic Engineering, University of Rome Tor Vergata, Rome, Netherlands*
Mummery, C.L. - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*
Mastrangeli, M. - *Department of Microeletronics, TU Delft, Netherlands*
Martinelli, E. - *Electronic Engineering, University of Rome Tor Vergata, Rome, Italy*
Van Meer, B.J. - *Anatomy, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Human pluripotent stem cell (hiPSC) derived cardiac cells (cardiomyocytes, cardiac endothelial cells and cardiac fibroblasts), provide great potential for the development of human cardiovascular models and for studying human heart development in vitro. Additionally, these differentiated cells have been widely used in the development of cardiac toxicity studies in vitro. Three-dimensional (3D) hiPSC-derived cardiac models, such as cardiac microtissues (MT) and engineered heart tissues (EHT), as well as cells grown in aligned scaffolds plates, have been shown to enhance maturation and to be superior in reproducing native tissue responses when compared to simple two-dimensional (2D) cultures. The aim of this study is to compare responses in contractility in the 4 different hiPSC-derived cardiac models after treatment with 12 different drugs previously selected for cardiac model qualification. The effect of each drug from the panel, at baseline and 4 different concentrations, were evaluated in the 4 different co-culture models at spontaneous beat rate and 3 different pacing frequencies. This large drug screening study allowed a head-to-head comparison of the different available hiPSC-derived cardiac models and a correlation of the effect of each drug concentration at each beating condition per model, giving us an insight on phenotypical differences and optimal conditions for functional experiments of each model. Additionally, this dataset was used as a base for the development of a more advanced contractility analysis software which allows more detailed segmentation of 3D tissues', the identification of individual contractile areas, and the construction of a complete contraction cycle diagram.

Funding Source: Project supported by Sartorius Stedim Biotech GmbH, Health~Holland (LSHM20018), NWO gravitation (024.003.001), The Novo Nordisk Foundation Center for Stem Cell Medicine (NNF21CC0073729) and Fujifilm Cellular Dynamics.
Keywords: hiPSC-derived cardiac models, Cardiotoxicity drug screening, hiPSC-derived cardiac contractility

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EXOSOMAL AND CELLULAR EXPRESSION OF LONG NON-CODING RNAS IN AN INDUCED PLURIPOTENT STEM CELL MODEL OF HYPERTROPHIC CARDIOMYOPATHY

Smith, James, *University of East Anglia, Norwich, UK*
Johnson, Ben - *MED, University of East Anglia, Norwich, UK*

Abstract: Hypertrophic Cardiomyopathy (HCM) is a form of genetic heart disease that affects the size, metabolism, calcium signalling and contractility of cardiomyocytes. This can lead to serious clinical outcomes including sudden cardiac death. Long non-coding RNAs (lncRNAs) are molecules of RNA >200 nucleotides in length. LncRNAs are not translated into protein, instead taking part in various cellular functions including signalling, regulation, chromatin modification and gene splicing. LncRNAs are expressed intracellularly and within exosomes, and many have been implicated in various cardiac diseases. The aim of this work is to investigate changes in cellular and exosomal levels of lncRNAs caused by HCM, and to uncover the role that target lncRNAs play in HCM pathogenesis. To achieve this, human iPSCs were reprogrammed from cells donated by a HCM patient (and a healthy relative) according to Smith et al., (2018). IPSCs were cultured in 2D monolayer and differentiated into cardiomyocytes according to methods adapted from Burridge et al., (2015). RNA was isolated from cells using Macherey-Nagel™ NucleoSpin™ RNA isolation. cDNA synthesis was performed using UltraScript RT (PCR Biosystems). Gene expression was analysed using RT-qPCR and the ddCt method. Exosomes were isolated using Exo-SpinTM columns (Cell Guidance Systems) or by ultacentrifugation. Exosome analysis was performed on the ExoView® platform (Nanoview Biosciences). The cellular expression of two lncRNAs (RMRP and SNORD116) is downregulated in iPSC-derived cardiomyocytes that have a HCM-causing mutation, compared to a healthy genetic relative. Conversely, these lncRNAs show greater enrichment in HCM iPSC-CM exosomes compares to the healthy control, a pattern that is further emphasised when the cardiomyocytes are subjected to electrical pacing to increase contraction frequency. Overall, HCM iPSC-CMs have a higher exosomal output compared to healthy iPSC-CMs, a trend which is further emphasised when the cells are subjected to stress. In conclusion, evidence suggests that lncRNAs play a role in the exosomal and cellular

signalling pathways that are involved in HCM. Further study is needed to establish what this role is and how they links to clinical outcomes of the disease.

Keywords: Hypertrophic Cardiomyopathy, lncRNA, Exosomes

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DIFFERENT EFFECTS OF BETAINE ON CARDIAC CELLS AND MESENCHYMAL STEM CELLS

Vaiciuleviciute, Raminta - *Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania*
Pachaleva, Jolita - *Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania*
Uzieliene, Ilona - *Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania*
Porvaneckas, Narunas - *Faculty of Medicine, Vilnius University, Vilnius, Lithuania*
Bernotiene, Eiva - *Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania*

Abstract: Betaine is a natural compound acting as an antioxidant, osmoprotectant and anti-inflammatory agent. Since betaine reduced oxidative stress and modulated metabolism of cardiac tissue, we were interested to investigate if the responses of other tissues are similar. The aim of our study was to compare the responses of human cardiomyocyte line AC16 and human bone marrow mesenchymal stem cells (BMMSCs) to betaine treatment under inflammatory conditions. AC-16 cell line was purchased from SigmaAldrich. Human mesenchymal stem cells were were isolated from bone marrow tissues, remaining after surgical procedures. Cell proliferation was analysed using alamarBlue™ dye under normal and inflammatory (with IL-1β) conditions with or without betaine (10 mM) pretreatment for 24 hours. Intracellular calcium (iCa2+) levels were evaluated in all cell types as determined by the iCa2+ specific fluorescent dye Cal-520. Morphologic parameters of cells were evaluated under 2 different doses of betaine (10 mM and 40 mM) using HoloMonitor®. The results showed contrary effect of betaine on AC16 and BMMSCs proliferation – it increased proliferation of AC16 cells while proliferation of BMMSCs tended to be downregulated. IL-1β reduced AC16 proliferation and betaine pretreatment significantly prevented this effect on day 1 while its longer incubation with betaine enhanced IL-1β induced decrease of proliferation. For BMMSC, on the contrary, betaine significantly diminished IL-1β induced rise of proliferation. ICa2+ analysis confirmed different cellular responses to betaine treatment, as it reduced iCa2+ levels in AC16 cells, while increased in BMMSCs. Both high and low dose of betaine treatment reduced migration of AC16 cells while BMMSCs responded to 40 mM betaine differently, as 10 mM betaine resulted in higher migration as compared to the control group. In summary. betaine differently modulates cellular responses in human cardiomyocytes and BMMSCs. Reduced proliferation under inflammatory conditions, iCa2+ level and migration suggests that betaine treatment has stabilising effect on AC16 cells, while in BMMSCs it showed stimulating results by attenuating effect of IL-1β, increasing iCa2+ level and cell migration.

Funding Source: This research was funded by European Union's Horizon 2020 research and innovation program under Grant Agreement No. 953138.
Keywords: Mesenchymal stem cells, Cardiomyocytes, Betaine

TOPIC: EARLY EMBRYO

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THE HUMAN TROPHOBLAST LINEAGE GIVES RISE TO EXTRA-EMBRYONIC MESENCHYME EMPHASIZING EARLY DEVELOPMENT AS A RICH SOURCE OF EVOLUTIONARY INNOVATION

Liu, Zukai - *Single Cell Biology Laboratory, The Jackson Laboratory For Genomic Medicine, Plainville, CT, USA*
Tan, Yuliana - *Single Cell Biology Laboratory, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*
Flynn, William - *Single Cell Biology Laboratory, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*
Oliveira, Nelio - *Cellular Engineering, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*
McDonough, Justin - *Cellular Engineering, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*
Skarnes, William - *Cellular Engineering, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*
Robson, Paul - *Single Cell Biology Laboratory, The Jackson Laboratory For Genomic Medicine, Farmington, CT, USA*

Abstract: As ontogeny does not recapitulate phylogeny and less developmental restraint exists prior to gastrulation, early human development differs significantly from other mammalian species. Understanding how it differs is key to directing stem cell differentiation, modeling peri-implantation biology, and defining the genomic loci contributing to these evolutionary differences. Temporal differences in the emergence of extra-embryonic mesenchymal cells (ExMC) have been noted with ExMC appearing much earlier in the human embryo than in the mouse. We utilized human induced pluripotent stem cells (hiPSC) to explore ExMC development. We initiated our studies on a well-documented model of hiPSC differentiation to the trophoblast lineage (BMP4 induction/FGF signal inhibition) that leads to large multinucleated cells producing copious chorionic gonadotropin within 6 days. Utilizing single cell RNA-seq, we first analyzed the cellular dynamics of this differentiation time-course. A relatively uniform cell population transitioned from pluripotency to the trophoblast lineage over the first 3 days, without expression of early mesoderm regulators. At Day 4, a lineage bifurcation began, and led to two distinct populations by Day 6, one of these was clearly trophoblast while the other, with strong mesenchymal properties, we define as ExMC. The ExMC cell identity was further confirmed by integrating our data with single cell datasets derived from in vivo post-implantation embryos of cynomolgus monkey and human. We next optimized media composition for exclusive ExMC induction and mined expression data to identify HAND1 as a potential upstream regulator of ExMC formation. A knockout of this transcription factor indicated comprehensive down-regulation of ExMC markers and up-regulation of trophoblast markers. These results demonstrate HAND1 is essential for ExMC specification from the trophectoderm lineage and drives a SNAI2-mediated epithelial-to-mesenchymal transition. The novel finding that ExMC can emerge from the trophoblast lineage clarifies long-standing confusion in the human PSC-derived extra-embryonic lineage literature, emphasizes species differences in early development, and provides an iPSC-derived model in which to further explore these evolutionary novelties highly relevant to human health.

Keywords: Extra-embryonic mesenchymal cell, human induced pluripotent stem cell, HAND1

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DEVELOPMENT OF A SYNTHETIC MODEL OF EARLY EMBRYONIC DEVELOPMENT USING CRISPR EPIGENOME EDITING IN EMBRYONIC STEM CELLS

Lodewijk, Gerrald - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Kozuki, Sayaka - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Cook, Nathan - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Topacio, Benjamin - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Shariati, Ali - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*

Abstract: Life begins with a fertilized egg that undergoes several divisions to generate more undifferentiated cells. This clump of cells then differentiates into 3 distinct cell types to form the blastocyst: 1) Epiblast cells, which later form all tissues in the embryo, characterized by expression of NANOG. 2) Hypoblast cells (GATA6+) and 3) trophoblast cells (CDX2+), which are the major source of all extra-embryonic tissues, such as the yolk sac and placenta to facilitate embryonic growth and implantation into the maternal uterus wall. It is observed that about 25-50% of human embryos fail to implant, however our understanding of human embryogenesis and implantation is limited due to the inaccessibility of the implanting embryo and ethical considerations. To address these limitations, we aim to further develop synthetic embryo models. Using doxycycline-inducible CRISPR-activation engineered mouse embryonic stem cell lines, we show efficient induction of fate determining transcription factors for epiblast (Nanog), hypoblast (Gata6) and trophoblast cells (Cdx2), followed by morphological changes in each cell type. Next, we assess how these cell lines can self-organize into early embryonic structures, using previously defined 2D micropatterning and 3D organoid models. The use of CRISPR-Cas9 engineered stem cell lines may provide new insights into self-organization mechanisms of such models, by allowing controllable assembly of precise numbers and types of cells. In future work, we aim to develop the same system in human embryonic stem cells, followed by comparative analysis and genome-wide perturbation studies between mouse and human embryonic model systems, to identify human-specific mechanisms in embryogenesis.

Keywords: Synthetic embryo models, CRISPR-dCas9 gene regulation, Stem cell engineering

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BIOPHYSICS AND MECHANOBIOLOGY OF LUMENOGENESIS IN HUMAN PLURIPOTENT STEM CELLS

Indana, Dhiraj - *Mechanical Engineering, Stanford University, Stanford, CA, USA*
Agarwal, Pranay - *Orthopaedic Surgery, Stanford Medicine, Stanford, CA, USA*
Bhutani, Nidhi - *Orthopaedic Surgery, Stanford Medicine, Stanford, CA, USA*
Chaudhuri, Ovijit - *Mechanical Engineering, Stanford University, Stanford, CA, USA*

Abstract: Embryoids or human pluripotent stem cell (hPSC) models of embryonic development, serve as an excellent tool to uncover mechanisms regulating early morphogenetic and differentiation events during human embryogenesis. Recently several embryonic tissues have been modeled in-vitro, but these models rely on poorly defined reconstituted basement membrane-based matrices such as Matrigel or use suspension culture which lacks cell-matrix interactions. Thus, the impact of matrix properties on hPSC self-organization in embryoids is unknown. One such embryoid model is that of the epiblast. The epiblast is formed in-vivo by a cluster of hPSCs which undergo polarization and lumen formation in response to extracellular matrix signaling. In this work, we use viscoelastic alginate hydrogels with independently tunable stress relaxation (a measure of viscoelasticity), RGD ligand density and stiffness to model the epiblast and show that matrix mechanics regulate hPSC lumenogenesis. Higher RGD density and fast stress relaxation promote lumen formation and apicobasal polarization while slow stress relaxation at low RGD density triggers hPSC apoptosis. Surprisingly, matrix stiffness did not significantly impact hPSC lumen formation. Pluripotency is maintained through day 14 of culture in all alginate formulations. This is in sharp contrast with 3D Matrigel culture where hPSCs form lumens but lose pluripotency after day 3 of culture. Modulating matrix stress relaxation provides control over lumen size, and cluster characteristics of hPSCs in fast relaxing, high RGD gels recapitulate epiblast nuclear and cluster morphology. These results highlight matrix viscoelasticity as a critical factor regulating stem cell morphogenesis and provide key insights into the role of mechanical cues during development of the human embryo. Next, we studied the biophysical mechanisms driving lumen formation in hPSCs. Lumen opening is found to be driven by a novel actin mediated, pressure independent mechanism, which switches to osmotic pressure driven growth once a critical lumen size is reached. Overall, our findings reveal a new lumenogenesis mechanism and advance our understanding of human peri-implantation development.

Funding Source: Stanford Bio-X Interdisciplinary Initiatives Program Seed Grant for Ovijit Chaudhuri and Nidhi Bhutani National Science Foundation Grant (CMMI 1846367) for Ovijit Chaudhuri
Keywords: lumen formation, epiblast, viscoelasticity

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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GENERATING BRAIN ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Lee, Junghyun - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
O'Connor, Erin - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Giadone, Richard - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Sauer, Michelle - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Holton, Kristina - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University,*

Cambridge, MA, USA
Engmann, Anne - *Department of Stem Cell and Regenerative Biology, and Center for Brain Science, and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Saxena, Monika - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Wells, Kaylee - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Gampierakis, Loannis - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Gasperini, Caterina - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Showkat, Nazaf - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Florido, Mary - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, and Harvard T.H. Chan School of Public Health, Cambridge, MA, USA*
Buchanan, Sean - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Macklis, Jeffrey - *Department of Stem Cell and Regenerative Biology and Center for Brain Science, and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*
Rubin, Lee - *Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*

Abstract: During development endothelial cells (ECs) migrate into the brain, where under the inductive influence of the brain environment, they differentiate into the cells that form the blood-brain barrier (BBB). Dysfunction of the BBB has been associated with cognitive impairment in aging and dementia. To carry out detailed studies of the molecular and cellular changes that accompany BBB dysfunction, a reliable in vitro brain EC model is essential. In order to differentiate human pluripotent cells (hPSCs) into brain ECs, we recapitulated the developmental trajectory of ECs in vitro. First, hPSCs were differentiated into peripheral ECs by mesodermal commitment followed by expansion and isolation of CD144-positive cells. Next, by modulating Wnt, TGF-beta, and STAT3 signaling pathways, peripheral ECs were converted to brain EC-like cells. In addition, overexpression of brain EC transcription factors, along with epigenetic modulation, further augmented brain EC phenotypes including upregulation of GLUT1, MFSD2A, ABCB1, CLDN5, downregulation of PLVAP, and decreased permeability. Our work provides an improved human brain EC model that can be used for understanding the development and maintenance of BBB properties in heath and disease.

Funding Source: This work was supported by Simons Foundation Plasticity and the Aging Brain Program, NINDS (1R01NS117407), NIA (1R01AG072086), and the Harvard Stem Cell Institute.
Keywords: Brain Endothelial cell, Blood Brain Barrier (BBB), in vitro model

TOPIC: EPITHELIAL_GUT

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ONE-STEP GENERATION OF TUMOR MODELS BY BASE EDITOR MULTIPLEXING IN ADULT STEM CELL-DERIVED ORGANOIDS

Gandhi, Shashank - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Geurts, Maarten - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Boretto, Matteo - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Hendriks, Delilah - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Celotti, Martina - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Schürmann, Paul - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*
Clevers, Hans - *Developmental Biology and Stem Cell Research, Hubrecht Institute, Utrecht, Netherlands*

Abstract: Recent optimization of CRISPR/Cas9-mediated genome engineering has resulted in the development of base editors that can efficiently mediate C>T and A>G transitions. Combining these genome engineering tools with human adult stem cell (ASC)-derived organoid technology holds promise for disease modeling. Here, we demonstrate the application of base editors for the generation of complex tumor models in human ASC-derived hepatocyte, endometrial and intestinal organoids. First, using conventional and evolved Cas9-variants, we show the efficacy of both cytosine and adenine base editors and use them to model four hot-spot point mutations in CTNNB1 in hepatocyte organoids. Next, we apply C>T base editors in endometrial organoids to insert nonsense mutations in PTEN and demonstrate tumorigenicity even in the heterozygous state. Furthermore, we use cytosine base editors for simultaneous oncogene activation (PIK3CA) and tumor-suppressor inactivation (APC and TP53). To increase the flexibility of base editor multiplexing, we then combine SpCas9 and SaCas9 base editors for simultaneous C>T and A>G editing at individual target sites. Finally, we show the power of base editor multiplexing by modeling colorectal tumorigenesis in a single step by simultaneously transfecting sgRNA's targeting four cancer genes.

Funding Source: Schmidt Science Fellowship
Keywords: CRISPR/Cas9, Organoids, Tumorigenesis

TOPIC: EPITHELIAL_LUNG

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MODELLING IDIOPATHIC PULMONARY FIBROSIS USING PATIENT-SPECIFIC HIPSC-DERIVED ALVEOLAR EPITHELIAL TYPE II CELLS FOR STUDYING RESPIRATORY INFECTIONS IN VITRO

Serna-Valverde, Ana Lilia - *Cancer and Stem Cells, University of Nottingham, UK*
Reed, Liam - *Cancer and Stem Cells, The University of Nottingham, UK*
Cuevas-Ocaña, Sara - *Cancer and Stem Cells, The University of Nottingham, UK*
Kiu Lo, Peggy Cho - *Cancer and Stem Cells, The University of*

Nottingham, UK
Tatler, Amanda - *Respiratory Medicine, The University of Nottingham, UK*
Jenkins, Gisli - *Respiratory Medicine, The University of Nottingham, UK*
Hannan, Nicholas - *Cancer and Stem Cells, The University of Nottingham, UK*

Abstract: Idiopathic pulmonary fibrosis (IPF) is a chronic, invariably fatal, interstitial lung disease with a median survival of 2-3 years. Its etiology is unclear; however, environmental and genetic risk factors indicate that alveolar epithelial type II (AEII) cell dysfunction plays a pivotal role. Mutations in genes for pulmonary surfactant production compromise AEII cell function and viability, reducing its capacity to regenerate. Furthermore, infection-induced lung injury has been related to AEII cell damage and disease progression. The activation of fibrogenic pathways by respiratory viruses can promote acute lung injury and has been associated with acute exacerbations of IPF, a leading cause of mortality. The study of this host-pathogen interaction is hampered by the lack of adequate human IPF models. Therefore, the differentiation of patient-derived human induced pluripotent stem cells (hiPSC) into alveolar organoids represents a valuable tool for studying genotype-phenotype relationships of IPF in vitro. For this, we created an in vitro organoid platform using patient-derived hiPSCs carrying a Surfactant Protein C (SFTPC) mutation and using CRISPR/Cas9 base-editing techniques a corrected, isogenic control. This directed differentiation recapitulates in vivo developmental pathways to generate lung progenitors which were matured in 3D-culture conditions to obtain a population of AEII cells with the capacity to express SFTPC. AEII cells were successfully enriched using fluorescent activated cell sorting using antibodies directed against carboxypeptidase M (CPM) and sodium-dependent phosphate transport protein 2B (NaPi2b), utilised as surface-markers for NKX2.1 and SFTPC, respectively. The obtained cells were exposed to H1N1 influenza A virus and samples were analysed using single-cell RNA sequencing (scRNA-seq) and proteomics. Following exposure, transcriptome profiling revealed many differentially expressed genes that contribute to the inflammatory response, fibrosis, and cell function. Our data suggest the model can be useful to identify host-pathogen response biological pathways underlying acute exacerbations of IPF, needed for the development of therapeutic strategies that limit the progression of fibrosis in response to infections.

Funding Source: Medical Research Council CONACYT University of Nottingham
Keywords: hiPSCs-derived organoids, Respiratory infection, Idiopathic Pulmonary Fibrosis

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INVESTIGATING BPIFA1 AND ITS ROLE IN REGULATING EPITHELIUM HOMEOSTASIS IN THE DEVELOPING HUMAN NEONATAL AIRWAY

McVicar, Rachael N. - *Biomedical, Sanford Burnham Prebys, La Jolla, CA, USA*
Leibel, Sandra - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Snyder, Evan - *Biomedical, Sanford Burnham Prebys, La Jolla, CA, USA*

Abstract: The human airway epithelium serves to protect the host from foreign pathogens and environmental insults. However, infants who are born prematurely have underdeveloped lungs with weakened barrier function, and insult is added to injury after the rapid transition from hypoxia in-utero to normoxia postnatal. Consequently, premature infants are at greater risk of severe respiratory syncytial virus (RSV) infections and developing chronic airway inflammation. One protein of interest, BPIFA1, has been shown to regulate airway epithelium homeostasis and prevent pathogen induced airway disease severity in adult mice models. While this protein has been rigorously studied for its role in regulating airway mucus viscosity as well as serving as an antimicrobial peptide during respiratory infections in mice, little research has been done investigating BPIFA1 in a developmentally compromised human neonatal airway. Therefore, we wish to investigate if endogenous BPIFA1 expression is influenced by changes in oxygen tensions, and if supplemental BPIFA1 decreases the severity of RSV infection in the preterm lung epithelium. First, the level of BPIFA1 expression was assessed in human stem cell (iPSC) derived and human fetal lung tissue derived airway epithelial cultures representing the preterm airway. Next, the influence of oxygen tension on BPIFA1 expression was investigated by culturing airway epithelial cells in either hypoxic (5% oxygen) or normoxic (21% oxygen) conditions; where an increase in BPIFA1 expression was observed in normoxic airway cultures. RNA was extracted from both hypoxic and normoxic airway cultures and differential gene expression is being assessed via bulk RNA sequencing under these differing oxygen states. Lastly, the antiviral potential of BPIFA1 is being investigated by infecting airway epithelial cultures with RSV to determine if supplementing recombinant BPIFA1 protein can decrease overall RSV infection. Our findings will help elucidate how premature birth impacts airway development and BPIFA1 expression and explore the therapeutic potential of exogenous BPIFA1 in the premature infant population.

Keywords: Airway Epithelium Modeling, Lung Homeostasis, Preterm Lung Development

TOPIC: EPITHELIAL_SKIN

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ESTABLISHING IN VIVO AND IN VITRO MODELS OF HYPERMOBILE EHLERS-DANLOS SYNDROME FOR VALIDATING CAUSATIVE MUTATIONS AND THERAPY DEVELOPMENT

Jesberg, Parker - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Denver, CO, USA*
Diette, Nicole - *Department of Biological and Physical Sciences, Keystone College, La Plume, PA, USA*
Velmurugan, Kalpana - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Escamilla, Jazmin - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Han, Chann Makara - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Rozhok, Andrii - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Pavlova, Maryna - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Kogut, Igor - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz*

Medical Campus, Aurora, CO, USA
Bilousova, Ganna - *The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: Ehlers-Danlos Syndrome (EDS) is an incurable genetic connective tissue disorder characterized by hyperextensible skin, joint hypermobility and cutaneous fragility. EDS is primarily caused by mutations in genes encoding collagens or proteins involved in collagen biogenesis. However, the genetic etiology for the most common form of EDS, hypermobility-type (EDS-HT), remains unknown. Using next generation sequencing, we have identified multiple missense mutations in the TNXB gene as likely candidates that cause EDS-HT in our cohort of patients. TNXB encodes the tenascin-X protein, which is known to participate in collagen assembly. Tenascin-X-deficiency is associated with classical EDS, while tenascin-x haploinsufficiency can result in EDS-HT. We suggest that our candidate mutations cause EDS-HT by either mimicking tenascin-x haploinsufficiency or acting in a dominant negative manner. To address this hypothesis, we focused on developing currently lacking in vivo and in vitro models of EDS-HT. We first established a skin xenograft model of EDS-HT by healing wounds inflicted on the backs of immunodeficient mice using human keratinocytes and EDS patient fibroblasts. The developed EDS skin xenografts showed disorganized collagen fibrils in the extracellular matrix, resembling those seen in skin biopsies of EDS-HT patients. To complement our in vivo model, we also developed an in vitro cell-based model of EDS-HT by generating 3D skin equivalents using fibrin gel scaffolds and EDS-HT human fibroblasts. Skin equivalents derived from EDS fibroblasts showed reduced collagen density and an increased rate of collagen damage upon mechanical stress consistent with the EDS-HT phenotype. Our in vivo and in vitro models can now be used to assess novel therapies for EDS and to validate candidate mutations in the TNXB gene as a cause of EDS-HT. We have already reprogrammed EDS fibroblasts into induced pluripotent stem cells (iPSCs). We are currently correcting these candidate mutations using CRISPR/Cas9. The corrected EDS iPSCs will be differentiated into fibroblasts and used in our in vivo and in vitro models. If the EDS-HT phenotype is no longer observed in our models, this will confirm that this candidate mutation is indeed the cause of EDS-HT in these patients.

Keywords: Ehlers-Danlos Syndrome, iPSCs, Tenascin-X

TOPIC: GERMLINE

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AN EFFICIENT AND SCALABLE METHOD TO GENERATE GERM CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Overeem, Arend - *Anatomy and Embryology, Leiden University Medical Centre, Amsterdam, Netherlands*
Chang, Yolanda - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Chuva de Sousa Lopes, Susana - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: In vitro gametogenesis (IVG) from human pluripotent stem cells (hPSCs) holds great potential for improving reproductive health. Achieving IVG will enable the modelling of infertility-causing diseases, and may ultimately help to generate innovative personalized treatments and even new assisted reproduction techniques. The first step of IVG consists of the specification of human primordial germ cell-like cells (hPGCLC) from hPSCs. Currently, protocols for the generation of hPGCLCs in vitro are based on 3D culture of cell aggregates in U-bottom wells. These methods are characterized by low differentiation efficiency, and suffer from poor scalability, making it difficult to obtain enough hPGCLCs for downstream optimization of IVG protocols. To address this issue, we have tested a variety of alternative hPGCLC differentiation methods with better potential for scalability. Based on this, we have developed a new 2D culture system that allows for the generation of hPGCLCs in high numbers in the absence of a feeder layer. This new method is not technically-challenging and the obtained hPGCLCs express hallmark germ cell markers, such as TFAP2C, POU5F1 and SOX17. We expect that the ability to generate high numbers of hPGCLCs will greatly facilitate researchers in the field of IVG to set-up comprehensive and high-throughput approaches, that will accelerate progress in the subsequent IVG differentiation steps necessary to ultimately generate functional gametes.

Funding Source: This work was supported by the Dutch Research Council (VICI-2018-91819642) and reNEW (Novo Nordisk Foundation)
Keywords: gametogenesis, germline, primordial germ cells

TOPIC: HEMATOPOIETIC SYSTEM

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INVESTIGATING THE FUNCTION OF WASP IN THROMBOPOIESIS AND PATHOGENIC MECHANISMS OF THROMBOCYTOPENIA OF WISKOTT-ALDRICH SYNDROME USING ISOGENIC PLURIPOTENT STEM CELL MODELS

Wang, Mengge - *BESE, King Abdullah University of Science and Technology (KAUST), Jeddah, Saudi Arabia*
Li, Mo - *BESE, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*
Yuan, Baolei - *BESE, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*
Zhou, Xuan - *BESE, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*

Abstract: Wiskott-Aldrich syndrome (WAS) is an X-linked recessive monogenic disease characterized by thrombocytopenia, severe immunodeficiency, eczema, and cancer predisposition. It is caused by mutations in the Wiskott-Aldrich syndrome protein, WASP. Although the role of WASP in terminally differentiated lymphocytes has been well characterized, how WASP regulates megakaryopoiesis and thrombopoiesis is poorly understood. Most megakaryocytes reside in the bone marrow, and performing invasive bone marrow biopsies in mostly pediatric WAS patients is undesirable. Meanwhile, there are over 200 mutations that lead to hypomorphic levels or complete loss of WASP, and it is impossible to predict clinical severity from the genotype. To avoid phenotype ambiguity due to mutational background and ascertain the true loss-of-function phenotype, we established isogenic WASP knockout human pluripotent stem cells (hPSCs) using the CRISPR/Cas9 technology and a megakaryocyte/platelet differentiation system. We took advantage of these isogenic models to dissect the role of WASP in the nucleus and cytoplasm of megakaryocytes. Our preliminary data show that WASP knockout impairs the formation of platelets which is in keeping with the clinical characterization of WAS and with the role of WASP in cytoskeleton organization. Unexpectedly, we also found the generation of

multi-polyploidy megakaryocytes is suppressed in WASP knockout cells. Furthermore, our preliminary data showed that WASP is not only expressed in the cytoplasm but also in the nucleus of megakaryocytes, which indicates that WASP may also participate in nuclear functions such as RNA splicing and the organization of nuclear compartments via liquid-liquid phase separation. Thus, the isogenic hPSC models of WAS will provide mechanistic insights into the normal function of WASP in megakaryopoiesis and elucidate pathogenic mechanisms of thrombocytopenia in WAS.

Keywords: Wiskott-Aldrich syndrome, pluripotent stem cell, thrombopoiesis

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3D BIOPRINTING USING SELF-ASSEMBLING TETRAMERIC PEPTIDE FOR AUTOMATED FABRICATION OF MULTICELLULAR ACUTE MYELOID LEUKEMIA DISEASE MODEL

Alhattab, Dana - *Biological, Environmental Science and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Alshehri, Salwa - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*
Isaioglou, Ioannis - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*
Khan, Zainab - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*
Susapto, Hepi - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*
Merzaban, Jasmeen - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*
Hauser, Charlotte - *Biological & Environmental Science & Engineering, KAUST, Thuwal, Saudi Arabia*

Abstract: Acute myeloid leukemia (AML) is a hematological malignancy that remains a therapeutic challenge due to the high incidence of disease relapse and drug resistance. In order to achieve a better understanding of disease relapse, improve current treatments and identify novel targeted therapies, robust preclinical models are essential. Such models should represent the three-dimensional (3D) spatial structure of the bone marrow (BM) microenvironment and accurately mimic its complexity. In this study, we aimed to achieve an automated fabrication of 3D AML disease models that truly recapitulates the biological complexity of the BM microenvironment and could be used in drug screening applications. To build our model, we have developed a unique class of tetramer self-assembling peptides with an innate ability to self-assemble in aqueous solutions into stable hydrogels. The model was built using the peptide hydrogel and incorporating leukemic cells, BM mesenchymal stem cells (BM-MSCs), and endothelial cells (ECs). Both cell types play essential roles in shaping the BM microenvironment and protecting AML cells. Additionally, using our in-house developed robotic bioprinter and a novel extrusion unit design, we achieved an automated fabrication of a 3D multicellular AML disease model Using our bioprinting system and the self-assembling peptide, we could efficiently 3D print leukemic cells, MSCs, and ECs into a multicellular 3D disease model. The peptide hydrogel formed a highly porous network of nanofibers with mechanical properties (stiffness) close to the BM extracellular matrix (ECM). The 3D peptide culture supported the viability and growth of leukemic cells, BM-MSCs, and ECs. Additionally, the 3D peptide-based multicellular culture recapitulated cell-cell and cell-ECM interactions. Besides, the 3D peptide-based multicellular culture induced a quiescent state and drug resistance in leukemic cells compared to other culture models. The developed 3D peptide-based multicellular culture provides a better model for drug screening and development; additionally, it could be used to perform investigational studies on the role of the tumor microenvironment in tumor development and drug resistance. In the future, we aim to use this model in personalized medicine applications to test patient-specific therapeutics.

Keywords: 3D disease model, Acute myeloid leukemia, Bioprinting

TOPIC: IMMUNE SYSTEM

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PLACENTAL MESENCHYMAL STEM CELLS BOOST M2 ALVEOLAR OVER M1 BONE MARROW MACROPHAGES VIA IL-1β IN KLEBSIELLA PNEUMONIAE-MEDIATED ACUTE RESPIRATORY DISTRESS SYNDROME

Wang, Li-Tzu - *Department of Obstetrics & Gynecology, National Taiwan University, Taipei, Taiwan (Republic of China)*
Yen, B. Linju - *Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes, Zhunan, Taiwan*
Wang, Hsiu-Huan - *Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes, Zhunan, Taiwan*
Chao, Ying-Yin - *Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes, Zhunan, Taiwan*
Lee, Wei - *Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes, Zhunan, Taiwan*
Huang, Li-Yueh - *National Institute of Infectious Diseases & Vaccinology, National Health Research Institutes, Zhunan, Taiwan*
Chiu, Sheng-Kang - *Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan*
Siu, L. Kristopher - *National Institute of Infectious Diseases & Vaccinology, National Health Research Institutes, Zhunan, Taiwan*
Liu, Ko-Jiunn - *National Institute of Cancer Research, National Health Research Institutes, Zhunan, Taiwan*
Sytwu, Huey-Kang - *National Institute of Infectious Diseases & Vaccinology, National Health Research Institutes, Zhunan, Taiwan*
Yen, Men-Luh - *Department of Obstetrics & Gynecology, National Taiwan University, Taipei, Taiwan*

Abstract: Acute respiratory distress syndrome (ARDS) is a lethal complication of severe bacterial pneumonia due to the inability to dampen over-exuberant immune responses without compromising pathogen clearance. Both of these processes involve tissue-resident and bone marrow (BM)-recruited macrophage (MΦ) populations which can be polarized to have divergent functions. Surprisingly, despite the known immunomodulatory properties of mesenchymal stem cells (MSCs), simultaneous interactions with tissue-resident alveolar MΦs (AMΦs) and recruited-BMMΦ populations are largely unexplored. In this study, we assessed the therapeutic use of human placental-MSCs (PMSCs) in severe bacterial pneumonia-related ARDS with elucidation of the roles of resident AMΦs and recruited BMMΦs. We developed a lethal,

murine pneumonia model using intratracheal infection of a clinically relevant serotype K2 strain of Klebsiella pneumoniae (KP) with subsequent intravenous human PMSC treatment. Phenotype and functionality of pulmonary AMΦs and recruited BMMΦs, histological evaluation, bacterial clearance, and mice survival were assessed. To elucidate the role of resident AMΦs in improving outcome, we performed AMΦ depletion in the KP-pneumonia model with intratracheal clodronate pretreatment. Human PMSC treatment decreased tissue injury and improved survival of severe KP-pneumonia mice by decreasing the presence and function of recruited M1 BMMΦ while preserving M2 AMΦs and enhancing their anti-bacterial functions. Interestingly, PMSC therapy failed to rescue AMΦ-depleted mice with KP pneumonia, and PMSC-secreted IL-1β was identified as critical in increasing AMΦ anti-bacterial activities to significantly improve pathogen clearance—especially bacteremia—and survival. In conclusion, human PMSC treatment preferentially rescued resident M2 AMΦs over recruited M1 BMMΦs with overall M2 polarization to improve KP-related ARDS survival.

Funding Source: This work was partially funded by the Taiwan Ministry of Science & Technology (109-2326-B-002-016-MY3 to L.T.W., 107-2314-B-002-104-MY3 and 110-2314-B-002-042 to M.L.Y.), and the NHRI (11A1-CSPP06 & CS-111-GP-01 to B.L.Y.).
Keywords: Placental-mesenchymal stem cells (PMSCs), Klebsiella pneumoniae (KP), Alveolar macrophages (AMΦs)

TOPIC: KIDNEY

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THE REGULATORY PROGRAM OF THE DISTALIZING MAMMALIAN NEPHRON

Achieng, MaryAnne A. - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Schnell, Jack - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Fausto, Connor - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Kim, Sunghyun - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Parvez, Riana - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
McMahon, Andrew - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Lindstrom, Nils - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: During development, nephron progenitors differentiate into over 24 cell-types that become positioned along the nephron's proximal-distal axis. These precursor-progeny relationships are poorly understood. To improve distal cell type differentiation in the in vitro organoid model, we delineated the developmental programs generating distal nephron cells in vivo by analyzing single cell omic (scRNA- and snATAC-seq) data capturing nephrogenesis in mice and humans. Gene expression profiles and putative regulatory regions are largely shared in murine and human distal nephron development, indicating deeply conserved regulatory programs, though species-specific expression and putative enhancers are also evident. More specifically, scrutiny of distal cell diversity in human nephrons show that transcription boundaries of loop of Henle nephron cell types are shared in mice and humans. Two distinct cortical thick ascending limb (cTAL) cell types can be defined in human nephrons by mutually exclusive expression of transcriptional repressors IRX1 and IRX3. Our trajectory models also show that the renin-regulating macula densa and the IRX1+ cTAL emerge from a common PAPPA2+ precursor population, with a clear branching event and distinct chromatin accessibility patterns. These analyses delineate how these terminal cell fate decisions are made in the distal nephron. Comparisons of in vivo data to organoid RNA-seq data from 2 well-established differentiation protocols highlighted poor organoid fidelity to in vivo cell expression profiles. Organoids show a lack of canonical markers of mature distal cell types and display asynchronous differentiation. To correct patterning abnormalities in organoids, we optimized kidney organoid differentiation in two iPSC lines and generated synchronized and uniform renal vesicle-like structures forming after 5 days in 3D organization. Further manipulation of β-catenin signaling by pulsing of small molecule inhibitors increased the abundance of distal precursors, partially normalized patterning, and increased expression of cell-markers associated with distalization and distal cell maturation. We present a platform for synchronized nephrogenesis suitable for studying patterning programs in the nephron and formation of functionally competent distal-like cells.

Keywords: distal nephron, kidney organoids, nephrogenesis

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HIGH-CONTENT PHENOTYPIC ANALYSES OF SYNCHRONIZED KIDNEY ORGANOID PATTERNING

Schnell, Jack - *Development, Stem Cells, and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Achieng, MaryAnne - *Development, Stem Cells, and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Fausto, Connor - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Lindström, Nils - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Congenital renal diseases are poorly understood as insufficient detail to developmental precursor-progeny relationships prevents linking developmental abnormalities to their specialized mature kidney cell derivatives. Strategies to generating novel regenerative therapeutics for kidney diseases are similarly impeded by an incomplete understanding of the developmental programs driving renal cell diversity. The most abundant cell in the kidney – proximal tubule cells – perform the bulk of renal reabsorption and are highly susceptible to injury. Defects to these cells often manifest as urinary wasting of nutrients. Though studies have identified transcription factor Hepatocyte nuclear factor 4 alpha (Hnf4a) as necessary for proximal tubule maturation, the developmental program for proximal tubule cells remain unclear. Holding human induced pluripotent stem cell-derived kidney organoids to the benchmark of in vivo development, our preliminary analyses of kidney and organoid single-cell transcriptomic data show consistent early developmental patterning defects and incomplete maturation of proximal cells across kidney organoid models. However, our experiments show that positional identities within organoid nephrons are sensitive to manipulation. Application of small-molecule inhibitors modify early patterning and positional identities and alters maturation of proximal cell lineages. Promoting the spatial and temporal expansion of the Notch ligand Jagged 1 (JAG1+) nephron domain increases the abundance of HNF4A+ proximal tubules, supporting the hypothesis that a JAG1+ domain presages HNF4A expression – as shown by in vivo data. By modulating early patterning to stabilize specific developmen-

tal programs, our studies are informing in vitro nephrogenesis strategies building regenerative therapeutics.

Keywords: proximal tubule, synchronized nephrons, kidney organoids

TOPIC: LIVER

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LIN28 CONTROLS CANCER INITIATION THROUGH DIRECT TRANSLATIONAL REGULATION OF TARGET MRNAS

Hsieh, Meng-Hsiung - *Children Research Institute, UTSW, Dallas, TX, USA*

Abstract: Whether RNA-binding proteins (RBPs) are modifiers or essential drivers of carcinogenesis is unknown. Furthermore, the critical targets and effector mechanisms of RBPs, which facilitate complex interactions within ribonucleoprotein (RNP) networks, are vague. We probed the Lin28 family of RBPs as a model system to address these questions in vivo. First, we found that Lin28 RBPs are essential for the initiation of chemical mutagen and RAS driven liver cancers. In the context of an aggressive NrasG12V/Tp53 KO mixed hepatocellular/cholangiocarcinoma (HCC/CCA) model, mice without Lin28a and Lin28b were unable to generate cancer, indicating a strict requirement for Lin28s in tumor initiation. Second, a doxycycline-induced Lin28a/b deletion model showed prevention of tumorigenesis induced by the mutagen diethylnitrosamine (DEN). Importantly, this powerful on-off model facilitated a rapid transgenic approach to discover genetic mechanisms through which Lin28s permit HCC initiation. We explored an RBP oncofetal network of 16 factors that are linked to Lin28 through mRNA and protein interactions. Interestingly, 15 of these 16 factors are RBPs, most of which are not regulated by let-7. Polysome profiling analysis showed Lin28B knockdown in HCC cells decreased translational activity on 8 of these RBPs. Moreover, in vivo rescue experiments showed that 8 of these RBPs could restore tumor formation in Lin28a/b deficient mice. These results suggest that Lin28 facilitates the formation of an RNP network that is essential for cancer initiation and could be a potential target for therapeutic prevention of liver cancer.

Keywords: Lin28, liver cancer, RNA binding protein

TOPIC: MUSCULOSKELETAL

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ENGINEERING A VASCULARIZED 3D BIOARTIFICIAL SKELETAL MUSCLE PRENATALLY USING AMNIOTIC FLUID STEM CELLS

Calà, Giuseppe - *Division of Surgery and Interventional Science, University College London, UK*
Sun, Yunyan - *Great Ormond Street Institute of Child Health, University College London, UK*
Scaglioni, Dominic - *Great Ormond Street Institute of Child Health, University College London, UK*
Beesley, Max - *Great Ormond Street Institute of Child Health, University College London, UK*
Lutman, Roberto - *Great Ormond Street Institute of Child Health, University College London, UK*
Gomez-Salinero, Jesus Maria - *Department of Medicine, Weill Cornell Medicine, New York, NY, USA*
Tedesco, Francesco Saverio - *The Francis Crick Institute, London, UK*
Rafii, Shahin - *Department of Medicine, Weill Cornell Medicine, New York, NY, USA*
De Coppi, Paolo - *Great Ormond Street Institute of Child Health, University College London, UK*
Gerli, Mattia Francesco Maria - *Division of Surgery and Interventional Science, University College London, UK*

Abstract: In vitro 3D models of human skeletal muscle are a powerful platform for investigating the molecular processes behind muscle diseases, regeneration, and the development of novel therapies. Amniotic fluid stem cells (AFSCs) are fetal stem cells that can be isolated from routine amniocentesis. They are broadly multipotent, making them an attractive source for autologous cell-based therapies and in vitro patient-specific modelling. To date, no study has reported the generation of 3D skeletal muscle tissue using AFSCs. Our aim is to engineer a vascularized skeletal muscle construct using a combination of human AFSCs and 3D extracellular matrix. AFSCs were isolated, characterized and reprogrammed into myogenic and endothelial cells by inducing the expression of MYOD and ETS transcription factors (ETV2, FLI1, ERG), respectively. AFSC-derived endothelial cells exhibit and maintain a vascular phenotype (CD31+, VE-Cad+, VEGFR2+) and form stable vascular networks in vitro. Similarly, AFSC-derived myogenic cells efficiently differentiate to myoblasts, and subsequently fuse to form MyHC+ myotubes in 2D culture. To increase structural complexity and create a physiologically relevant in vitro model, we generated 3D skeletal muscle constructs by combining AF-derived myogenic cells in a fibrin hydrogel. Following differentiation, these 3D skeletal muscle constructs display formation of MyHC+ multinucleated myotubes, spatially aligned along the longitudinal axis. Moreover, they exhibit typical mature skeletal muscle structural and functional proteins such as Desmin and Dystrophin. Overall, this work demonstrates that human AFSCs can be efficiently induced towards different lineages with minimal manipulation and can be utilised for the generation of high-fidelity skeletal muscle tissue. AFSC-derived endothelial and myogenic cells can be combined within 3D structure to enable tissue vascularization and facilitate integration with host vasculature should these constructs be used for implantation. 3D engineered skeletal muscles represent a versatile tool for modelling muscle disorders in vitro. Importantly, these constructs could function as building blocks for developing novel autologous translational therapies for conditions such as congenital malformations and volumetric muscle loss.

Funding Source: Our work is supported by an EC H2020 Marie Skłowdoska-Curie Individual Fellowship, GOSH BRC, UCL Division of Surgery, British Heart Foundation.
Keywords: amniotic fluid stem cells, skeletal muscle engineering, 3D

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ANCESTRY-INFORMED HUMAN INDUCED PLURIPOTENT STEM CELLS USED TO ELUCIDATE THE MOLECULAR MECHANISMS UNDERPINNING EWS/FLI TRANSFORMATION IN EWING SARCOMA

Becklin, Kelsie L. - *Pediatrics, University of Minnesota, Minneapolis, MN, USA*
Mills, Lauren - *Bioinformatics, University of Minnesota, Minneapolis, MN, USA*

Moriarity, Branden - *Pediatrics, University of Minnesota, Minneapolis, MN, USA*
Moss, Rachel - *Bioinformatics, University of Minnesota, Minneapolis, MN, USA*
Spector, Logan - *Epidemiology, University of Minnesota, Minneapolis, MN, USA*
Thue, Nicole - *Pediatrics, University of Minnesota, Minneapolis, MN, USA*
Webbewr, Beau - *Pediatrics, University of Minnesota, Minneapolis, MN, USA*

Abstract: Ewing Sarcoma (ES) is a rare but deadly pediatric bone and soft tissue tumor. Survival has improved little in decades despite identifying the driving fusion oncoprotein EWS-FLI1. EWS-FLI1 is a notably toxic, aberrant transcription factor that induces growth arrest and apoptosis in most cell types. ES occurs at higher rates (roughly 10-fold) in children of European (EUR) ancestry compared to those with primarily African (AFR) ancestry. Ancestry is in fact the strongest risk factor for ES, but the molecular basis of this disparity is unclear. To explore this, we obtained induced pluripotent stem cell (iPSC) lines from multiple individuals each of ~100% EUR and AFR ancestry, and multiple iPSC lines with intermediate EUR/AFR admixture (45%-90% AFR). To evaluate differential tolerance to EWS/FLI based on ancestry, we differentiated iPSC from each ancestry into neural crest cells (iNCC), a proposed cell-of-origin for ES, and expressed either EWS/FLI1-2A-GFP or GFP-only control vectors using lentivirus. We monitored each line for GFP expression by flow cytometry over the course of 21 days to determine tolerance to EWS/FLI across ancestries. The GFP control vector was maintained at a high frequency (~95%) regardless of ancestry, whereas EWS/FLI1-2A-GFP+ cells were progressively lost from the populations in agreement with the known toxicity of EWS/FLI1. Strikingly, iNCC derived from 100% EUR lines maintained a significantly higher frequency of EWS/FLI1-GFP+ cells compared to 100% AFR lines, with iNCC of admixed ancestry showing intermediate tolerance. To investigate the molecular basis of this observation across ancestries, we evaluated global gene expression changes induced by EWS-FLI1 using RNA-seq, as well as genome-wide occupancy of EWS/FLI1 using CUT&TAG. EWS-FLI1-induced pronounced changes to the transcriptome, with 13,578 differentially expressed genes (adjusted p-value < 0.05) compared to controls. Of these, 3,128 genes exhibited ancestry-associated differences in gene expression in response to EWS-FLI1, illuminating novel biologic pathways underlying ES tumorigenesis. Further analysis is ongoing and updated results will be presented. Our study demonstrates the feasibility and utility of ancestry-informed iPSC modeling to identify novel and potentially targetable pathways to treat ES.

Keywords: Cancer modeling, Ewing sarcoma, Ancestry Informed iPSC

TOPIC: NEURAL

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USING HIPSC-DERIVED CHOROID PLEXUS ORGANOIDS TO ASSESS THE ROLE OF SURFACTANT PROTEINS IN NEONATAL INTRAVENTRICULAR HEMORRHAGE (IVH) & POST-HEMORRHAGIC HYDROCEPHALUS (PHH)

Carmona Mendoza, Andrelia - *Biology, California State University, San Marcos, Escondido, CA, USA*

Abstract: Intraventricular hemorrhage (IVH) is a common complication in preterm newborns, particularly at early gestational ages (GA) & low birth weight. Post-hemorrhagic hydrocephalus (PHH) frequently develops following IVH. ~20% of neonates born < 27 weeks GA suffer from IVH; ~25% develop PHH. Premature neonates are also prone to acute respiratory distress syndrome (RDS) because they are born prior to the developmentally-timed production of pulmonary surfactant which is dependent on the developmentally-timed synthesis of surfactant proteins (SPs) in the lungs. Not only does RDS result, but also hemodynamic instability which perturbs cerebral blood flow, both posited to contribute to IVH. We & others have found SPs – surprisingly – to be present in periventricular ependymal cells of the brain (based on our study of hiPSC-derived cerebral organoids & primary human fetal brain tissue), suggesting that they may be pivotal to the integrity of the CSF-blood barrier & fluid dynamics. We hypothesize that IVH may not only be a secondary consequence of RDS, but may also be tied, as a primary etiological factor, to the developmental lag in SP production in the periventricular region. We have generated choroid plexus organoids from hiPSCs from a patient born with an SP Type B (SP-B) deletion, & from CRISPR-corrected isogenic control hiPSCs to help evaluate SP-B's role in the pathogenesis of IVH. We've established a developmental timeline of SP-B expression within our organoids (validated using primary human fetal brain). We've provided further validation by assessing SP-B production in fetal monkey brains of different GAs. Data-to-date suggest the presence of SPs (A, B, & C) & the co-localization of the water regulation protein Aquaporin 1 & the tight junction proteins Zona Occludens-1 & Claudin 5 in the ependymal lining of the ventricle-like cavities of the organoids. These findings suggest the SPs are present at this physiological interface & may contribute to barrier integrity; conversely, their absence could predispose to IVH & PHH. We are preparing to identify a developmental proteomic profile of SPs in vivo models of IVH & PHH. Establishing this unanticipated role of cerebral SPs in the pathogenesis of IVH & PHH may suggest novel therapies in the care of premature newborns.

Keywords: hydrocephalus, ogranoids, hiPSC

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UNDERSTANDING APOE4-MEDIATED SUSCEPTIBILITY TO TAU-RELATED COGNITIVE IMPAIRMENTS IN THE MIBRAIN MODEL

R'Bibo, Lea - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Forton-Juarez, Ana - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Mesentier-Louro, Louise - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Frank, Alexander - *Neuroscience, Icahn School of Medicine at*

Mount Sinai, New York, NY, USA
Blanchard, Joel - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract: Tau pathology, characterized by hyperphosphorylation and aggregation of Tau to neurofibrillary tangles (NFTs), correlates better with cognitive outcomes in Alzheimer's disease (AD) than beta-amyloid plaques severity, shifting the focus of AD research. Interestingly, APOE4, the strongest genetic risk factor for non-familial AD, is associated with increased severity of human postmortem Tau pathology compared to carriers of the most common APOE3 allele, and APOE4 carriers have a higher risk of chemotherapy-induced cognitive impairments (CBI). We hypothesize that similarly to mice models, human brain tissue from APOE4 carriers is susceptible to more severe tau pathology during disease development and progression, an effect that is driven through cell type specific mechanisms. To test this hypothesis, we developed the multi-cellular integrated human brain (miBrain) model, which recapitulates key brain tissues through the co-culturing of all major brain cell types derived from human induced pluripotent stem cells (iPSC). Tau pathology can be induced in the miBrain by recapitulating Tau seeding events through treatments with Tau fibrils or increasing endogenous Tau phosphorylation using the chemotherapeutic methotrexate. In line with mouse and clinical studies, APOE4/4 miBrain cultures exhibit increased Tau pathology compared to isogenic APOE3/3 miBrain. Using the high tractability of the miBrain model, subtraction and combinatorial genetic mixing experiments revealed that the increase of Tau phosphorylation observed in this APOE4 human brain tissue model is dependent upon the presence of APOE4 microglia. RNA-sequencing of these isogenic APOE3/3 and APOE4/4 microglia revealed differentially regulated signaling pathways. We are currently using chemical and genetic approaches to empirically validate the effect of specific pathways on the propagation of Tau pathology in APOE4 brain tissue. Unraveling the molecular mechanisms underlying this cell-specific effect of APOE4 on Tau pathology will pave the way for the development of new, more specific and highly efficient therapeutic approaches for AD adapted to a large number of patients.

Funding Source: Cure Alzheimer's Foundation, NIH/NINDS UG3NS115064-01
Keywords: Alzheimer's disease, APOE4, Tau

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THE AUTISM-ASSOCIATED GENE SYNGAP1 MODULATES HUMAN CORTICAL NEUROGENESIS

Del Dosso, Ashley N. - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Birtele, Marcella - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Wilkinson, Brent - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Nguyen, Tuan - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Xu, TianTian - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Taitano-Johnson, Christopher - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Coba, Marcelo - *Psychiatry and Behavioral Sciences, University of Southern California, Los Angeles, CA, USA*
Quadrato, Giorgia - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Advances in human genomics have dramatically accelerated our understanding of the genetics of neurodevelopmental disorders (NDDs), including autism spectrum disorders (ASD). De novo mutations in a variety of synaptic protein genes have been reported in individuals with ASD, making it a prototypical synaptopathy. However it is important to characterize the role that classically defined synaptic protein genes have at different developmental stages with longitudinal modeling in a human cellular context. Here we utilize a human induced pluripotent stem cell-derived cortical organoid model to dissect the role of SYNGAP1, a Synaptic GTPase Activating Protein, which is amongst the highest confidence risk factors for ASD. For the first time, we reveal the expression of SYNGAP1 in human radial glia progenitors (hRGPs). We show that SYNGAP1 is highly expressed within the apical domain of hRGPs lining the wall of the developing ventricular zone. In a cortical organoid model of SYNGAP1 haploinsufficiency, we show a dysregulation in the filamentous actin dynamics of the hRGP. Disruption of cytoskeleton dynamics impairs the scaffolding and the division plane of the hRGPs ultimately resulting in disruption of cortical plate lamination and in accelerated cortical neurogenesis. Overall, our work reframes our understanding of the impairments in neural circuit function observed in SYNGAP1 patients by connecting it not only with the well-known alteration in synaptic transmission, but also with early developmental defects. Finally, this discovery highlights the importance of unraveling the stage specific function of genes associated with NDD to uncover new avenues for therapeutic interventions.

Funding Source: USC Stem Cell Baster SynGAP Research Fund
Keywords: SYNGAP1, Brain Organoid, Neurodevelopment

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PROTEOMIC ALTERATIONS AND NOVEL MARKERS OF NEUROTOXIC REACTIVE ASTROCYTES IN HUMAN IPSC MODELS

Labib, David - *The New York Stem Cell Foundation, New York, NY, USA*
Wang, Zhen - *Departments of Structural Biology and Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN, USA*
Prakash, Priya - *Neuroscience Institute, NYU Grossman School of Medicine, New York, NY, USA*
Zimmer, Matthew - *The New York Stem Cell Foundation Research Institute, New York, NY, USA*
Smith, Matthew - *Department of Neurology, Johns Hopkins University, Baltimore, MD, USA*
Frazel, Paul - *Neuroscience Institute, NYU Grossman School of Medicine, New York, NY, USA*
Barbar, Lilianne - *The New York Stem Cell Foundation Research Institute, New York, NY, USA*
Sapar, Maria - *The New York Stem Cell Foundation Research Institute, New York, NY, USA*
Calabresi, Peter - *Department of Neurology, Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD, USA*
Peng, Junmin - *Departments of Structural Biology and Developmental Neurobiology, Center for Proteomics and Metabolomics, St. Jude Children's Research Hospital, Memphis, TN, USA*
Liddelow, Shane - *Neuroscience Institute, Department of Neuroscience & Physiology, Department of Ophthalmology and*

Parekh Center for Interdisciplinary Neurology, NYU Grossman School of Medicine, New York, NY, USA
Fossati, Valentina - *The New York Stem Cell Foundation Research Institute, New York, NY, USA*

Abstract: Astrocytes, the most abundant macroglial cell type in the central nervous system, respond to inflammation, injury, and infection by acquiring reactive states in which they may become dysfunctional and contribute to disease pathology. Human induced pluripotent stem cell (hiPSC) modeling has been critical to uncover the roles of astrocytes in multiple neuroinflammantory and neurodegenerative diseases such as Alzheimer's disease and multiple sclerosis. We have previously established a differentiation protocol to generate hiPSC-derived astrocytes and induce in vitro a reactive sub-state driven by proinflammatory factors TNF, IL-1α, and C1q ('TIC'), which has been shown as a source of neurotoxicity. Here, we investigated the surface marker profile and proteome of TIC-induced reactive astrocytes. A flow cytometry screen identified VCAM1, BST2, ICOSL, HLA-E, PD-L1, and PDPN as putative, novel markers of this reactive sub-state. Notably, we found that several of these markers colocalize with GFAP+ cells in post-mortem samples from people with Alzheimer's disease. Moreover, we performed whole cell proteomic analysis of TIC-induced reactive astrocytes and detected proteins and related pathways primarily linked to potential recruitment of and engagement with peripheral immune cells. Taken together, our findings will serve as new tools to purify reactive astrocyte subtypes to further explore their roles and their involvement in immune responses. Our overall goal is to identify potential molecular targets for pharmacological interventions that prevent the deleterious effects of neuroinflammation in neurodegenerative diseases.

Funding Source: The New York Stem Cell Foundation; NINDS; NIA; The Cure Alzheimer's Fund; The Alzheimer's Association; The Blas Frangione Foundation; Alzheimer's Research UK; and NYU Grossman School of Medicine
Keywords: reactive astrocytes, inflammation, neurodegenerative diseases

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NOVEL DIFFERENTIATION PROTOCOL TO GENERATE FUNCTIONAL HUMAN NOCICEPTORS FOR TRANSLATIONAL DISEASE MODELING

Kalia, Anil Kumar - *Institute of Physiology, RWTH Aachen University, Aachen, Germany*
Rösseler, Corinna - *Institute of Physiology, RWTH Aachen University, Aachen, Germany*
Truong, Vincent - *Anatomic Incorporated, Minneapolis, MN, USA*
Walsh, Patrick - *Anatomic Incorporated, Minneapolis, MN, USA*
Lampert, Angelika - *Institute of Physiology, RWTH Aachen University, Aachen, Germany*

Abstract: Human induced pluripotent stem cells (hiPSCs) can differentiate into any cell type of the body. Nociceptors are specialized sensory neurons involved in the detection and transmission of potentially painful stimuli from periphery to higher brain areas. Clinically, peripheral sensory neuropathies can result in complete loss of pain or severe painful neuropathic symptoms. hiPSC-derived nociceptors are a potentially scalable source of human tissue that may overcome limitations in using primary tissues. They can provide an important platform to study pain disorders and potentially discover novel pain therapeutics. In this study, we characterized a sensory neuron population manufactured from hiPSCs using a novel directed differentiation methodology. Multiple hiPS clones derived from different reprogramming methods, genetics, age, and somatic cell source were used to generate sensory neurons. Inherited erythromelalgia (IEM) and Small fiber neuropathy (SFN) patient derived hiPS cell lines were used to elucidate the disease-causing mechanisms. Patch clamp technique was applied to characterize the electrophysiological phenotype of neurons. We found that this protocol resulted in the differentiation of iPSCs to immature neurons within 7 days as compared to 10 days or more with conventional methods. Highly dense and homogenous neuronal networks of sensory neurons were obtained without the use of mitomycin C to suppress non-neuronal outgrowth. Immunofluorescence staining confirmed the expression of Peripherin and Tuj1 marker proteins. Patch clamp recordings indicate mature action potentials by day 28 of maturation. We also confirmed the expression of the sodium channel subtype Nav1.8 in these neurons with a subtype selective Nav1.8 blocker. Diseased iPS derived neurons displayed cellular hyperexcitability and reduced rheobase as compared to control groups, indicating that this protocol may also be used for modeling disease in a dish. This study demonstrates reliable differentiation of hiPSCs from multiple different donors into nociceptors which can be used for disease modeling. This method of patient-specific generation of neurons provides a source of nociceptors from varying genetic backgrounds that could help enable future discovery of novel pain therapeutics.

Keywords: Pain, Sensory Neurons, iPSCs

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MODELING X-LINKED DYSTONIA PARKINSONISM USING PATIENT DERIVED NEURONS

Aryal, Shivani - *Department of Developmental Biology, Washington University, Saint Louis, MO, USA*
Chen, Shawei - *Department of Developmental Biology, Washington University in St. Louis, MO, USA*
Yoo, Andrew - *Department of Developmental Biology, Washington University in St. Louis, MO, USA*

Abstract: X-linked Dystonia-Parkinsonism (XDP) is an adult-onset neurodegenerative disorder. It is inherited in an X-linked recessive pattern, with disease onset around the age of 40 years and characterized by marked loss of striatal medium spiny neurons (MSNs). XDP patients inherit seven disease specific mutations, all within the genomic region containing TAF1. To understand the underlying mechanism of XDP, we use human MSNs derived by direct conversion of patient fibroblasts using neurogenic microRNAs, miR-9/9* and -124 and MSN subtype-defining transcription factors CTIP2, MYT1L, DLX1 and DLX2. MSNs from direct reprogramming method maintain the age signature of starting fibroblasts, which is critical to understand adult-onset disorders. Here, we present our study in modeling XDP via neuronal reprogramming and our efforts in profiling MSNs derived from symptomatic XDP patients including neurodegenerative phenotypes and transcriptome dysregulation. Our results show that XDP patient fibroblasts can be successfully reprogrammed into MSNs and the patient-derived MSNs (XDP-MSN) display several degenerative phenotypes including neuronal death, DNA damage, and mitochondrial dysfunction compared to MSNs reprogrammed from age- and sex-matched control individuals. To further infer cellular state altered in XDP-MSNs, we carried out transcriptomic and chromatin accessibility analyses using RNA and ATAC sequencing. Our results so far indicate pathways related to neuronal function as well as movement disorders implicated in XDP-MSNs. Pathway analysis also revealed TNFa and NFKB pathway

as an upstream regulator. XDP-MSNs show significant increase in cell death compared to Control-MSNs in the presence of TNFa suggesting increased vulnerability to stress in XDP-MSNs.

Funding Source: Collaborative Center for X-Linked Dystonia-Parkinsonism
Keywords: Neurodegeneration, Reprogramming, Medium Spiny Neurons

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MODELING FRAGILE X SYNDROME WITH HUMAN BRAIN ORGANOIDS

Xu, Jie - *The Graduate Program in Genetics and Molecular Biology, Emory University, Atlanta, GA, USA*
Jin, Peng - *Department of Human Genetics, Emory University, Atlanta, GA, USA*
Kang, Yunhee - *Department of Human Genetics, Emory University, Atlanta, GA, USA*
Li, Yujing - *Department of Human Genetics, Emory University, Atlanta, GA, USA*
Wen, Zhexing - *Department of Psychiatry and Behavioral Sciences, Cell Biology, Neurology, Emory University, Atlanta, GA, USA*
Zhou, Ying - *Department of Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA, USA*

Abstract: Fragile X syndrome (FXS), a leading genetic cause of intellectual disability and autism spectrum disorder (ASD), is typically caused by CGG trinucleotide repeat expansion within the 5' UTR of the FMR1 gene that leads to gene silencing and a complete loss of its encoding protein – fragile X mental retardation protein (FMRP). FMRP is a selective RNA-binding protein that forms a messenger ribonucleoprotein complex with polyribosomes in the regulation of protein synthesis. Previous animal studies have identified a number of potential therapeutic compounds, some of which have been further tested in clinical trials. Yet, improvements of behavioral and cognitive outcomes in patients in these clinical trials have unfortunately been unsuccessful, suggesting 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. Here we use a 3-D human-specific model featuring neurodevelopment – human induced pluripotent stem cell (hiPSC)-derived brain organoids – to investigate the functional and molecular impact of FMRP deficiency in human brain development. We observed that FMRP deficiency led to dysregulated neurogenesis, neuronal maturation, neuronal excitability, as well as altered gene expression profile and cell-type-specific developmental trajectory in FXS forebrain organoids. We also found that inhibition of the phosphoinositide 3-kinase pathway, but not the metabotropic glutamate pathway disrupted in the FXS mouse model, could rescue the developmental deficits in FXS forebrain organoids. Using eCLIP-seq, we were able to identify a large number of human-specific mRNAs bound by FMRP, including CHD2, a well-known risk gene associated with intellectual disability and ASD. To further investigate the impact of FMRP deficiency on hippocampal development, we have recently developed FXS hippocampal organoids. Interestingly, we observed increased hippocampal NPC proliferation and decreased generation of PROX1+ dentate gyrus granule cells caused by the loss of FMRP. Our study thus has not only demonstrated the brain region-specific effects of FMRP deficiency, but also 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
Keywords: fragile X syndrome, FMRP, brain organoids

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MICROPLASTICS EXPOSURE AFFECTS NEURAL DEVELOPMENT OF HUMAN PLURIPOTENT STEM CELL-DERIVED CORTICAL SPHEROIDS

Li, Yan - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*
Hua, Timothy - *Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA*
Kiran, Sonia - *Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA*
Sang, Qing-Xiang Amy - *Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA*

Abstract: Plastics have been part of our ecosystem for about a century and their degradation by different environmental factors produce secondary microplastics. These microplastics have a size < 5mm and can enter in living organisms through inhalation and ingestion. They have been seen to accumulate in organs like brain, lung, and liver of animal species and even have been observed in the placenta of pregnant women. However, the effects of microplastics on human have not been well investigated. Therefore, to understand the possible effects of polystyrene-microplastics (PS-MPs) on human brain development, a 3D model of human forebrain cortical spheroids/organoids has been derived, which mimics the early developmental stage of human cerebral cortex. To analyze the effects of PS-MPs, the spheroids were exposed to 100, 50, and 5 µg/mL concentrations of 1 µm and 10 µm size PS-MPs during day 4-10 and day 4-30. The short-term microplastic exposure for early neural progenitors showed the promoted proliferation and higher gene expression of Nestin, PAX6, ATF4, HOXB4 and SOD2. For long-term exposure, flow cytometry results showed that the reduced cell viability. The genes of cellular stress, DNA damage, reactive oxygen response, cell proliferation, development, and patterning genes indicate PS-MP size- and concentration-dependent effects. In particular, β-tubulin III, Nestin, and TBR1/TBR2 gene expression decreased in PS-MP treated conditions compare to the untreated control. The results of this study suggest that the size- and concentration-dependent exposure to PS-MPs can adversely affect embryonic brain-like tissue development in forebrain cerebral spheroids/organoids. This study has significance in assessing environmental factors in neurotoxicity and degeneration in human systems.

Funding Source: Florida State University CRC grant, Pfeiffer Professorship for Cancer Research, and an Endowed Chair Professorship (to QXS), Florida Department of Health (9LA01 to QXS & YL); and National Science Foundation (1652992 to YL)
Keywords: microplastics, human pluripotent stem cells, cortical organoids

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MICROGLIA-INCORPORATED CEREBRAL ORGANOIDS ENABLE DISCOVERY OF MODULATORS OF THE NEUROIMMUNE AXIS IN COMPLEX BRAIN DISEASES

Skibinski, Gaia - *Biology, Herophilus, Inc., San Francisco, CA, USA*
Stanton, Morgan - *Research and Development, Herophilus, San Francisco, CA, USA*
Hegde, Sharath - *Research and Development, Herophilus, San Francisco, CA, USA*
Kato, Saul - *Research and Development, Herophilus, San Francisco, CA, USA*
Skibinksi, Gaia - *Research and Development, Herophilus, San Francisco, CA, USA*

Abstract: There is significant evidence of a central role of neuroinflammation in neurodegeneration. Modulation of microglia function is a promising therapeutic approach across a variety of neurodegenerative diseases. Existing human 2D cellular models do not adequately capture non-cell autonomous effects of microglia and resultant dynamic changes in brain phenotypes. Complex human 3D in vitro models, which capture the cellular diversity of the brain, may allow the deeper study of relevant biological processes in response to genetic and pharmacological perturbations. To study the role of microglia in neurodegeneration, we developed a Neuroimmune Cerebral Organoid (NI-CO) model by optimizing a protocol to stably integrate iPSC-derived microglia into iPSC-derived cerebral organoids. We show that microglia integrated into our NI-CO model display in-vivo-like morphology, spatial distribution and transcriptomic profiles. TREM2 mutations are associated with several neurodegenerative disorders and disrupt microglia function, including phagocytosis. We generated a TREM2 NI-CO model by stably integrating TREM2 deficient iPSC-derived microglia into cortical organoids. Loss of TREM2 elicited changes in microglia function, including migration, phagocytosis, morphology and activation state, and importantly, led to the display of non-cell autonomous pathophysiology in the cortical organoid tissue. We performed a phenotypic compound screen to identify hit molecules that rescue phagocytosis in TREM2 deficient iPSC-derived microglia. We then characterized the broader systems-level effects of these hit molecules using our NI-CO TREM2 model to understand their ability to rescue both microglia function and neuron-astrocyte pathophysiology. Our study demonstrates the utility of a scaled NI-CO model to holistically study brain pathophysiology and discover novel druggable disease pathways and drug candidates which normalize the dysfunctional neuroimmune axis in neurodegenerative diseases.

Funding Source: Herophilus, Inc.
Keywords: Organoid, Neuroimmune, Microglia

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MAPPING THE MOLECULAR AND CELLULAR COMPLEXITY OF NEURODEVELOPMENTAL DISORDERS WITH BRAIN ORGANOIDS

Wang, Lu - *Department of Neurosciences, University of California, San Diego, CA, USA*
Sievert, David - *Department of Neurosciences, UCSD, San Diego, CA, USA*
Gleeson, Joseph - *Department of Neurosciences, UCSD, San Diego, CA, USA*

Abstract: Human brain organoids (hBOs) self-assembled from human induced pluripotent stem cells (hiPSCs), can reproducibly model early brain development and molecular logistics highlight their advantage in investigating the pathogenesis of neurodevelopmental disorders (NDDs). Genetic causes for NDDs are associated with thousands of individual genes or gene clusters spanning a wide range of biological functions. The abnormalities of NDDs at cellular level during human brain development remain unclear, a systematic study that phenotypically links NDDs to the cellular abnormality in hBOs is absent. Leveraging a large recessive NDDs hiPSCs library including families with microcephaly, epilepsy, polymicrogyria, autism, and broad mental retardation in our lab, we generated patient-specific hBOs for over 20 families, including 50 individual iPSC lines. Immunostaining across these hBOs yielded a 75% successful modeling ratio, revealing specific cell types correlating to patients' clinical reports. Whole-exome sequencing (WES) with single-cell transcriptomics generated from these 20 families capturing over 1009282 cells demonstrated cell type specific developmental abnormalities for each of the NDD categories. Specifically, we found that the basal radial glia cells implicated in microcephaly, intermediate progenitor cells in polymicrogyria, excitatory neuron progenitors in epilepsy or autism. These cell type specific developmental abnormalities associated with a convergence of cell type-specific transcriptome alterations. DNA barcoding lineage tracing of the progenitor cells in patient hBOs point to the cellular origins of the dysfunctional cells in each of the NDD categories further validated the cell type specific manner for each of NDDs. Our study establishes a phenotypic atlas of NDDs which reveals the convergence of disease-specific cellular defects across a wide variety of mutations and genetic backgrounds.

Funding Source: Our study is supported by grant no. R01NS106387 and HHMI to J.G.G
Keywords: Neurodevelopment disorders, Brain Organoids, Multi-Omics lineage tracing

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GBA N370S AND LRRK2 G2019S IPSC-DERIVED DOPAMINE NEURONS SHOWED DEFICIT IN DOPAMINE PATHWAY AND DEVELOPED ALPHA-SYN AGGREGATION COMPARABLE TO SNCA A53T

Fathi, Ali - *Research and Development, Fujifilm Cellular Dynamics Inc., Madison, WI, USA*
Bakshy, Kiranmayee - *Discovery Research, Fujifilm Cellular Dynamics Inc., Madison, WI, USA*
Hilcove, Simon - *Marketing, Fujifilm Cellular Dynamics Inc., Madison, WI, USA*
Liu, Jeanie - *Research and Development, Fujifilm Cellular Dynamics Inc., Madison, WI, USA*

Abstract: Human iPSC-derived Dopaminergic (DA) neurons offer a developmentally and physiologically relevant in-vitro model of human midbrain DA neurons. DA neurons are midbrain projection neurons that innervate different regions in the CNS including the forebrain and striatum. Loss of these neurons causes dropping in dopamine levels in the CNS and results in multiple disorders including Parkinson's disease (PD) and dementia. DA neurons declining with age and are selectively vulnerable to the oxidative stress generated by the dopamine oxidation. Here we generated DA neurons from two PD patient iPSCs with either an inherited GBAN370S or LRRK2 G2019S mutation, or genetically engineered SNCAA53T iPSCs along with its isogenic apparently

healthy normal (AHN) iPSCs. Our results showed that GBAN370S and LRRK2 G2019S derived DA neurons produced and released significantly more dopamine compared to the AHN and SNCAA53T mutant DA neurons and have higher mitochondrial ROS and alpha-synuclein aggregation comparable to the SNCAA53T DA neurons. All mutant DA neurons showed lower GBA activity, and their neuronal activity decreased over time in the MEA assay compared to the AHN DA neurons. Thus, our results demonstrate that this panel of iPSC-derived DA neurons manifest many key disease related features and can be used as a reliable in-vitro model for dopaminergic neurons degeneration.

Funding Source: This study was funded by Fujifilm Cellular Dynamics Inc.
Keywords: Dopaminergic neurons, Neurodegeneration, Parkinson's disease

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EXAMINING THERAPEUTIC MECHANISMS OF ATXN2 KNOCKOUT IN IPSC-DERIVED MOTOR NEURON MODELS OF TDP-43 PROTEINOPATHY

Gill, Stanley P. - *Stem Cell and Regenerative Biology, Harvard University/Stem Cell Institute, Boston, MA, USA*
Eggan, Kevin - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*

Abstract: Transactive Response DNA Binding Protein 43 (TDP-43) is a ubiquitously expressed nucleic acid transport and processing factor that is essential for normal cellular development and function. Disruptions in TDP-43 function, particularly in the form of re-localization and aggregation in the cytoplasm, have been associated with the development of several neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). Approximately 97% of ALS patients will develop hyper-phosphorylated, hyper-ubiquitinated inclusions of TDP-43 in spinal cord motor neurons. Previous studies in mouse models of TDP-43 overexpression, wherein mice develop ALS-like symptoms and TDP-43 cytoplasmic aggregates in spinal cord motor neurons similar to humans, have shown that Ataxin-2 (ATXN2) knockout was sufficient to extend life span, mitigate the severity of symptoms, and reduce the burden of TDP-43 cytoplasmic aggregates in mice overexpressing TDP-43. However, the mechanism by which ATXN2 knockout confers this therapeutic potential has yet to be fully explained in a human system. Given that ATXN2 and TDP-43 are known interacting partners at the protein and RNA levels, we hypothesized that ATXN2 knockout may modulate the metabolism of TDP-43 protein or RNA in a therapeutic fashion. In this study, we used human stem cell-derived motor neurons to examine the effect that ATXN2 knockout conveys on TDP-43 RNA and protein metabolism in the context of TDP-43 nuclear overexpression and cytoplasmic restriction. We also explored the effect of cellular stresses such as oxidative and proteotoxic stress on the health and morphology of these engineered neurons to determine if ATXN2 knockout and/or dysregulated TDP-43 have any broader effects on how human motor neurons respond to disruptive forces that are commonly seen in an aging brain. We believe that exploring how ATXN2 knockdown reduces motor neuron degeneration will provide a path to developing new therapies for ALS and other diseases where TDP-43 proteinopathy plays an important role.

Funding Source: Research reported in this publication was supported by the National Institute Of Neurological Disorders And Stroke of the National Institutes of Health under Award Number F31NS122138.
Keywords: iPSC-derived Neuronal Models, Neurodegenerative Disease, Engineered iPSC Disease Models

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EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO SENSORY NEURONS

Knock, Erin - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Moosa, Alym - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
LeBlanc, Noemie - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: Sensory neurons (SNs) are responsible for conveying sensations like pain and temperature. Pain studies mostly rely on dissection of primary rodent dorsal root ganglia, which may be poor predictors of human pain response. To meet the need for a human-relevant pain model, we developed STEMdiff™ Sensory Neuron Differentiation Kit, which promotes differentiation of human pluripotent stem cells (hPSCs) to SNs via a neural crest cell (NCC) intermediate. Undifferentiated hPSCs were dissociated and plated at 2 x 105 cells/cm2 on hESC-qualified Corning® Matrigel®-coated plates in complete STEMdiff™ Neural Crest Differentiation Medium. After 6 days of incubation, the resulting NCCs were dissociated and plated at 2 x 105 cells/cm2 in complete STEMdiff™ Sensory Neuron Differentiation Medium. After 6 days of differentiation, SNs were matured by switching to complete STEMdiff™ Sensory Neuron Maturation Medium. After 6 days in maturation medium, the cells were fixed and immunostained for SN markers BRN3A, peripherin, TRPV1, and Nav1.7. STEMdiff™ Sensory Neuron Differentiation Medium consistently converts human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines maintained in mTeSR™1 (5 cell lines), TeSR™-E8™ (2 cell lines), and mTeSR™ Plus (3 cell lines) into BRN3A+ SNs (25.3% ± 6.9%; mean ± SEM; N=7 cell lines). SNs were plated in a 48-well multielectrode array (MEA) plate to measure activity and response to temperature increase and capsaicin treatment. hPSC-derived SNs display spontaneous neuronal activity with a mean firing rate (MFR) of up to 2.3 ± 0.4 Hz (mean ± SEM) on day 33. They also display an increase in MFR in response to an increase in temperature to 42°C for 5 minutes (27 ± 4 fold increase in MFR; mean ± SEM, N=3) or 100 nM capsaicin (25 ± 3 fold increase in MFR; mean ± SEM, N=3) on day 21. In summary, STEMdiff™ Sensory Neuron Differentiation Kit supports generation of functional SNs that display the expected pain stimulus response.

Funding Source: STEMCELL Technologies is a private for profit biotechnology company
Keywords: sensory neurons, pain, differentiation

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ENERGY IMBALANCE WITHIN VAPB P56S MOTOR NEURONS IN ALS TYPE VIII

Landry, Curran A. - *Genetics and Genome Sciences, Case Western Reserve University, Cleveland Heights, OH, USA*
McMonagle, Brianna - *Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA*
Wong, Garrett - *Pediatrics, University of California San Diego, CA, USA*
Mitne-Neto, Miguel - *Research and Development, Fleury Institute, Sao Paolo, Brazil*
Zatz, Mayana - *Genetics, University of Sao Paolo, Brazil*
Muotri, Alysson - *Pediatrics, UCSD, San Diego, CA, USA*
Miranda, Helen - *Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA*

Abstract: Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is the most common adult–onset motor neuron disease. Cases where there is a known family history of the disease are known as familial ALS (fALS), while those without are known as sporadic ALS (sALS). sALS make up roughly 90% of cases, however fALS has larger percentage of genetic association. One of the known causative mutations in fALS is in an autosomal dominant c.166C>T mutation in the VAPB gene which leads to a change of a proline to a serine at position 56 (P56S) in the VAPB/C protein and is the causative mutation for ALS Type VIII (ALS8). VAPB appears to play a role in many cellular processes having been shown to have roles ranging from tethering the mitochondria and the ER to autophagy. Our group uses patient derived-iPSCs to generate motor neurons and investigate ALS pathogenesis associated to VAPB(P56S). Preliminary data from our lab indicates a link between VAPB(P56S) and motor neuron homeostasis. VAPB(P56S) and VAPB knockout iPSC-derived motor neurons display electrophysiological dysregulation in vitro, when analyzed by a multielectrode array (MEA) system using the Maestro machine (Axion Biosystems). Neuronal activity is an energy demanding process, therefore we recently interrogated the mitochondrial function of VAPB(P56S) iPSC-derived motor neurons, using the JC-1 mitochondrial dye and the Nanocellect WOLF flow cytometer, to assess mitochondrial membrane potential (MMP). We observed a 1.4-fold increase in cells fluorescing green (indicating a decrease in the MMP), in VAPB(P56S) iPSC-derived motor neurons when compared to familial controls. Moreover, we generated VABP knockout iPSCs that we transduced with lentivirus expressing VAPB under a tetracycline inducible promoter. We performed an MTT assay and identified a requirement of VAPB expression for motor neuron progenitor cell expansion but not motor neuron maturation. Taken together, our data suggest that VAPB(P56S) not only disrupts motor neuron homeostasis but may play a role in neurodevelopment.

Funding Source: This publication was made possible by an NIH-funded predoctoral training grant to Curran Landry (T32 NS077888)
Keywords: VAPB, ALS, Neurodegeneration

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DYNAMIC 3D COMBINATORIAL GENERATION OF HPSC- DERIVED NEUROMESODERMAL ORGANOIDS WITH DIVERSE REGIONAL AND CELLULAR IDENTITIES TO INVESTIGATE NEURODEVELOPMENTAL DISORDERS

Whye, Dosh - *Neurobiology, Human Neuron Core at Boston Children's Hospital, Boston, MA, USA*
Wood, Delaney - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*
Kim, Kristina - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*
Chen, Cidi - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*
Makhortova, Nina - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*
Buttermore, Elizabeth - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*
Sahin, Mustafa - *Neurobiology, Boston Children's Hospital, Boston, MA, USA*

Abstract: Neuromesodermal progenitors represent a unique, bipotent population of progenitors residing in the tail bud of the developing embryo, which give rise to the caudal spinal cell types of neuroectodermal lineage identity as well as the adjacent paraxial somitic cell types of mesodermal origin. Modeling genetic disorders of the nervous system has largely been accomplished through the use of transgenic animal models; however, the advent of stem cell technologies such as induced pluripotent stem cells has allowed for direct investigation of human patient-specific pathological mechanisms. In vitro stem cell derived models of neuromesodermal progenitors have been generated by several groups to interrogate mechanisms of development in human embryogenesis, but seldom have they been used to model pathogenic mechanisms associated with neurodevelopmental disorders. Further, differentiation formats that have been constructed are largely primitive in nature (i.e. 2D monolayer) with limited directionality in cell fate patterning. We present a dynamic 3D combinatorial method to generate robust populations of neuromesodermal organoids, derived from human pluripotent stem cells, which possess the pliability to be directed along various cell trajectories as well as co-develop in a hybrid cellular organoid model. By employing a combinatorial screening approach, we were able to dissect critical pathways contributing to the expression of direct germ lineages such as neuroectodermal and mesodermal specification. Further, we examined the influence of timing, duration, and concentration of a major developmental signaling pathway on specifying & enhancing early neuromesodermal identity as well as downstream cell fate trajectories during later stages of differentiation. In addition, we explored the synergistic activities of known morphogens, present in the developing embryo, and their influence on regional patterning & lineage specification. These approaches have led to the development of a dynamic in vitro system for cellular organoid modeling that can be applied to the investigation of neurodevelopmental disorders, such as the neural tube defect Spina Bifida and the pediatric neuromuscular disorder Spinal Muscular Atrophy.

Funding Source: The Human Neuron Core is supported by the Rosamund Stone Zander Translational Neuroscience Center at Boston Children's Hospital.
Keywords: NEUROSCIENCE., INDUCED PLURIPOTENT STEM CELLS., NEURODEVELOPMENTAL.

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DEVELOPMENT AND CHARACTERIZATION OF AN IN VITRO MODEL OF SSADH DEFICIENCY USING PATIENT IPSC-DERIVED NEURONS TO SUPPORT UNBIASED SCREENING OF NOVEL THERAPEUTIC APPROACHES TO TREATMENT

Afshar Saber, Wardiya - *Neurology, Harvard Medical School, Boston, MA, USA*
Teaney, Nicole - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Sundberg, Maria - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Jumo, Hellen - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Buttermore, Elizabeth - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Winden, Kellen - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Pearl, Phillip - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Ebrahimi-Fakhari, Darius - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Sahin, Mustafa - *Neurology, Boston Children's Hospital, Boston, MA, USA*

Abstract: Succinic semialdehyde dehydrogenase (SSADH) deficiency is an autosomal-recessive neurometabolic disorder caused by bi-allelic mutations in the ALDH5A1 gene. It is the most prevalent inherited disorder of GABA metabolism and is characterized by accumulation of two neuromodulators, GABA and GHB (gamma-hydroxybutyric acid), in the CNS. Previous studies using rodent models have shown that disruption in GABA signaling can lead to dysregulation of mitochondria numbers, turnover, and function. Over the last 30 years, an expanded understanding of pathophysiology based on the corresponding animal model (Aldh5a1-/- mice) has emerged but effective pharmacotherapy remains elusive. Alternative models and therapies that address the accumulation of GABA and GHB, and their downstream effects, are needed. In collaboration with the Human Neuron Core at the Boston Children's Hospital, five clinically-phenotyped patients and unaffected sex-matched parents have been consented and recruited from our SSADH deficiency registry. Fibroblasts have been collected for reprogramming. Three iPSC patient lines and sex-matched parental controls have been generated at the Harvard Stem Cell Institute. We have established the first in vitro model of SSADH Deficiency based on iPSC-derived neurons. We successfully generated GABAergic and excitatory neurons based on transcription factor programming and characterized these models in respect to SSADH deficiency phenotypes such as GABA levels and mitochondria function. Additionally, we performed functional assays to investigate neuronal excitability based on optogenetics and calcium imaging in co-cultures of GABAergic and excitatory neurons to evaluate epileptiform activity in SSADH deficient iPSC-derived neurons and create cell-based models suitable for drug screening.

Keywords: iPSC-derived GABAergic neurons, Optogenetics, SSADHdeficiency

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ATP13A2 LOSS OF FUNCTION MUTATIONS IN MIDBRAIN ORGANOIDS REVEAL ASTROCYTES' CRITICAL ROLE IN PARKINSON'S DISEASE PATHOGENESIS

Coccia, Elena - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Morrone Parfitt, Gustavo - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Sarrafha, Lily - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Vangheluwe, Peter - *Cellular and Molecular Medicine, KU Leuven, Belgium*
Ahfeldt, Tim - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Blanchard, Joel - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, it has a complex etiology in which an interplay of environmental and genetic factors leads to loss of dopaminergic neurons (DN). Even though the degeneration is selective for DN in the substantia nigra, it is becoming clear that other cell types, such as astrocytes, are also altered and have an essential contribution to neurodegeneration. The mechanisms behind the pathology onset and progression are poorly understood, but the familial cases, caused by highly penetrant genetic variants, point to lysosomal and mitochondrial pathway disfunction. ATP13A2 loss of function genetic variants cause early-onset PD and Kufor-Rakeb syndrome, with an age of onset around 15yo. ATP13A2 was recently established as a lysosomal exporter of polyamines, which have protective anti-oxidant and autophagy regulator roles. The transporter dysfunction disrupts cellular polyamine distribution, induces lysosomal dysfunction and cell death. We differentiated isogenic hPSC lines harboring ATP13A2 into midbrain organoids and analyzed quantifiable phenotypes in whole organoids, DN and midbrain patterned astrocytes. We found that ATP13A2 variants deplete the DN population in the organoid, and alter astrocytic functions, which would further damage neuronal populations. Astrocytes presented increased activation, impaired lysosomal activity, and consequently, clearance capacity and α-synuclein accumulation. Overall, we developed a model that recapitulates hallmark signatures of PD in vitro enabling future studies and screens to elucidate and target the mechanisms of PD pathogenesis. We discovered that ATP13A2 has a critical role in astrocytic lysosomal function highlighting astrocytes as a new therapeutic target in PD.

Keywords: Parkison's Disease, Astrocytes, Neurodegeneration

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A COMBINED MULTI-OMICS AND BIOINFORMATICS APPROACH TO DEVELOP PRENATAL THERAPY IN DOWN SYNDROME

Lee, Sarah E. - *Center for Precision Health Research, National Human Genome Research Institute/ National Institutes of Health (NIH), Bethesda, MD, USA*
Morris, Samuel - *Center for Precision Health Research, National Human Genome Research Institute/ National Institutes of Health (NIH), Bethesda, MD, USA*
Mosley, Iman - *Center for Precision Health Research, National Human Genome Research Institute/ National Institutes of Health*

(NIH), Bethesda, MD, USA
Bianchi, Diana - *Center for Precision Health Research, National Human Genome Research Institute/ National Institutes of Health (NIH), Bethesda, MD, USA*
Guedj, Faycal - *Center for Precision Health Research, National Human Genome Research Institute/ National Institutes of Health (NIH), Bethesda, MD, USA*

Abstract: Down syndrome (DS) is caused by trisomy of human chromosome 21 (T21). Triplication of the more than 200 protein-coding genes mapped to chromosome 21 results in genome-wide gene expression dysregulation. Here, we used multi-omics (microarrays, single-cell RNA sequencing, and proteomics) in nine T21 individual-derived iPSCs and neural progenitor cells (NPCs) and age-sex matched controls to identify dysregulated genes and pathways in DS. We then interrogated the Connectivity map (Cmap) and Library of Integrated Network-Based Cellular Signatures (LINCS) databases to screen for potential drug candidates that can rescue global gene expression dysregulation in T21 derived NSCs. We hypothesize that T21 NPCs will replicate early brain development defects at the molecular and cellular levels and that the Cmap and LINCS can objectively identify novel treatments to rescue those defects. Microarray analysis identified only two DEGs shared between cell types, including HPLN1 and GBX2. Pathway analysis of microarray and proteomics data demonstrated that, compared to euploid controls, T21 NPCs showed more dysregulated pathways, particularly cell cycle regulation, DNA damage, and repair checkpoints versus iPSCs in which transcriptional regulation and Gα12/13 were the top dysregulated molecular networks. Principal component analysis revealed that T21 NPCs were highly variable, suggesting the importance of using single-cell RNA sequencing to better understand the molecular mechanisms underlying the DS phenotype. This approach identified additional dysregulated pathways in T21 NPCs including, EIF2 signaling, oxidative phosphorylation, sirtuin pathway, and RHOGDI pathway. Using several cell-based assays, we validated the cell-cycle deficits identified by the omics data. On average, T21 NPCs exhibited proliferation delays compared to Eup. We evaluated twenty-four compounds identified by CMAP and LINCS databases for toxicity and efficacy in the rescue of cell cycle defects in T21 NPCs and identified “hit” compounds for further preclinical studies. Candidate genes and pathways identified by omics approaches suggest targets and can be used with computational tools to identify therapeutic interventions that were confirmed in vitro.

Funding Source: NATIONAL HUMAN GENOME RESEARCH INSTITUTE 1ZIAHG200399-05
Keywords: Down syndrome, neurodevelopmental models, prenatal therapy

TOPIC: PANCREAS

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DEVELOPMENT OF AN IN VITRO MODEL OF STEM CELL-DERIVED ISLETS TO INVESTIGATE ISLET STRESS

Maestas, Marlie - *Division of Endocrinology, Metabolism & Lipid Research, Washington University, Saint Louis, MO, USA*
Veronese-Paniagua, Daniel - *Division of Endocrinology, Metabolism & Lipid Research, Washington University in St. Louis, MO, USA*
Augsornworawat, Punn - *Division of Endocrinology, Metabolism & Lipid Research, Washington University in St. Louis, MO, USA*
Maxwell, Kristina - *Division of Endocrinology, Metabolism & Lipid Research, Washington University in St. Louis, MO, USA*
Millman, Jeffrey - *Division of Endocrinology, Metabolism & Lipid Research, Washington University in St. Louis, MO, USA*

Abstract: Diabetes is a chronic disease characterized by loss of blood glucose control due to death or dysfunction of pancreatic insulin-producing beta cells. During the onset and progression of diabetes, beta cells have been shown to undergo endoplasmic reticulum (ER), inflammatory, and other types of cellular stresses resulting in beta cell death and/or dysfunction. Therefore, maintaining homeostasis within the islet is crucial for proper beta cell function. While some studies have used primary human β cells for studying cellular health and stress, this is often used as confirmation of specific findings in murine models. Recently, stem cell-derived islets (SC-islets) have not only become a potential renewable source for diabetes cell replacement therapy, but also a viable model system for studying islet health. However, employing SC-islets as a stress model has not previously been developed. Here, we utilized a combination of pharmaceutical and biologically relevant compounds to induce inflammatory, ER, and/or Golgi stress to SC-islets and primary human islets. During stress in SC-islets we observe increases in genes associated with the unfolded protein response (UPR), inflammation, and/or Golgi stress. We also see significant decreases in beta cell identity genes; these results are recapitulated in our primary human islets. To test the viability of the SC-islets we quantified caspase3/7 activity and our results indicate increased apoptosis in SC-islet and primary human islet under stressed conditions. Considering that current human studies are limited due to the patient-to-patient variability and non-renewable nature of primary human islets, our findings validate SC-islets as a robust in vitro model to better define the connection between diabetes pathology and stress.

Funding Source: T32GM139774-01
Keywords: Endoplasmic Reticulum Stress, stem-cell-derived islets, Inflammatory stress

TOPIC: PLURIPOTENT STEM CELLS

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MODELLING TREACHER COLLINS SYNDROME IN hiPSCs TO INVESTIGATE CLINICAL VARIABILITY

Hsia, Gabriella - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*
Nani, Diogo - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*
Alegria, Thiago - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*
Zechi-Ceide, Roseli - *Department of Clinical Genetics, Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo, Bauru, Brazil*
Kokitsu-Nakata, Nancy - *Department of Clinical Genetics, Hospital for Rehabilitation of Craniofacial Anomalies, University of São Paulo, Bauru, Brazil*
Netto, Luis Eduardo - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*
Kobayashi, Gerson - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*
Passos-Bueno, Maria Rita - *Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil*

Abstract: Treacher Collins syndrome (TCS) is a rare craniofacial disorder characterized by hypoplasia of facial bones, cleft palate, and ear abnormalities, mainly caused by loss-of-function variants in TCOF1. Which is important for production of rRNA and DNA damage response particularly during early embryonic development. In mice, loss of Tcof1 leads to apoptosis at the neural plate border associated with high levels of reactive oxygen species (ROS) and impairment in ribosome biogenesis, compromising neural crest cell emigration and leading to the craniofacial malformations. A still debatable question is the etiological mechanisms responsible for the large intra- and interfamilial clinical variability in TCS. Here, we investigate if mitochondrial metabolism and redox processes are deregulated in TCS, which in turn can be related to clinical variability and may open venues for prevention and treatment. With the use of induced pluripotent stem cells (iPSCs) and iPSC-derived neural plate border-like cells (NBCs), from 5 clinically different TCS patients and 3 controls, we show that, under oxidative stress (treatment with H2O2), TCS iPSCs exhibit higher levels of apoptosis, p53 and Caspase-3 expression compared to controls. Conversely, when TCS iPSCs and NBCs were treated with antioxidant (GSH) and p53 inhibitor (pifithrin-α) there was partial phenotype rescue. These preliminary results show that the present strategy recapitulates the TCS cellular phenotypes observed in animal models and suggest involvement of redox processes in TCS pathophysiology, and a correlation between TCS clinical severity and cell phenotype observed in culture. This in vitro model should enable further investigation of TCS clinical variability and screening for putative molecules aimed at treatment or prevention.

Funding Source: This project is funded by: FAPESP (2018/21706-2 and CEPID: 2013/08028-1), CAPES (1799645) and CNPq (305405/2011-5).
Keywords: Craniofacial Syndromes, induced Pluripotent Stem Cells (iPSCs), Redox Processes

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SCREENING FOR GENETIC ENHANCERS OF CAR-INK CELL MEDIATED TUMOR KILLING

Hassan, Mwaka M. - *Biology, California State University, San Marcos, San Diego, CA, USA*

Abstract: Adoptive transfer of cellular immunotherapies is a promising approach to cancer treatment, but several challenges impede broad availability. Fate Therapeuticshas developed a unique induced pluripotent stem cell (iPSC) based platform that enables mass production of multiplexed engineered off-the-shelf cell therapy products for on-demand patient access in a cost-effective manner. Several chimeric antigen receptor(CAR) expressing NK cell products derived from genetically modified iPSCs (CAR-iNK cells) have been developed with this platform andare currently under investigation in phase I clinical trials. Previous work has allowed us to enhance the therapeutic profile of our CAR-iNK cell products by including three engineered modifications. These include an IL15 receptor fusion (IL15RF) protein to enhance persistence, high-affinity non-cleavable CD16 (hnCD16) to augment antibody-dependent cellular cytotoxicity (ADCC) against multiple tumor targets, and CD38 knock-out for improved metabolic profile and to prevent anti-CD38 antibody mediated fratricide. In this study, we aim to identify additional potential genetic modifications called NK cell enhancers (NCEs) that we hypothesize can improve the ability of CAR-iNK cells to target tumor heterogeneity and kill challenging tumor cells.The NCE candidates were engineered into iPSCs expressing a CAR targeting the MICA/B pan-tumor antigen and controlled by a tetracycline-inducible system to help identify the ideal timings of gene expressions. Engineered iPSC lines were differentiated into iNK cells using a proprietary differentiation platform that produces uniform population of NK cells. Functional killing assays in the presence of doxycycline identified NCEs that showed increased killing of MICA/B+ CaSki and Calu-3 tumor cell lines compared to uninduced CAR-iNK cells. Further functional analyses including assessment of in vivo tumor control will be performed on selected NCEs to further evaluate their potential to be included in the next generation of off-the-shelf CAR-iNK cell products.

Keywords: CAR-iNK, immunotherapy, cancer

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PANCREATIC DYSFUNCTION DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS WITH MITOCHONDRIAL DNA MUTATIONS

So, Seongjun - *Department of Biomedical Science and Department of Medical Science, CHA University and University of Ulsan College of Medicine, Seongnam-si, Korea*
Lee, Yeonmi - *Center for Embryo and Stem Cell Research, CHA Advanced Research Institute, Seongnam-si, Korea*
Han, Jongsuk - *Department of Biomedical Science, CHA University, Seongnam-si, Korea*
Kang, Soonsuk - *Center for Embryo and Stem Cell Research, CHA Advanced Research Institute, Seongnam-si, Korea*
Choi, Jiwan - *Center for Embryo and Stem Cell Research, CHA Advanced Research Institute, Seongnam-si, Korea*
Kim, Bitnara - *Department of Biomedical Science, CHA University, Seongnam-si, Korea*
Kim, Song Cheol - *Department of Medical Science, University of Ulsan College of Medicine, Seoul, Korea*
Kang, Eunju - *Department of Biomedical Science & Center for Embryo and Stem Cell Research, CHA University & CHA Advanced Research Institute, Seongnam, Korea*

Abstract: Diabetes mellitus (DM) is a serious metabolic disease characterized by high blood sugar levels over a prolonged period due to insufficient insulin or impaired insulin sensitivity. In recent years, the prevalence of DM is increasing worldwide, and many studies have been conducted on cell therapy using human induced pluripotent stem cells (iPSCs) as DM therapeutic potential. However, it is not fully understood how mitochondrial genome (mtDNA) abnormalities affect the function of pancreatic cells differentiated from iPSCs. In this study, we established iPSC clones from peripheral blood mononuclear cells (PBMCs) of a patient with type 2 DM (T2D) and investigated mtDNA mutations using the Illumina Miseq platform. Then, we selected wild-type (5% mt-857G>A in the rRNA region) and mutant (100% mt6678A>G and 13% mt7970G>A mutations in the non-syn protein-coding region) iPSC clones and differentiated them into pancreatic cells. INSULIN, a pancreatic cell marker, was observed in both clones-derived pancreatic cells by immunocytochemistry. We investigated the mitochondrial respiration capacity in differentiated pancreatic cells with a seahorse platform. It was confirmed that oxygen consumption rates were significantly lower in the mutant. We next analyzed insulin production and glucose-stimulated insulin secretion using enzyme-linked immunosorbent assay (ELISA). The mutant pancreatic cells exhibited decreased insulin production and impaired insulin secretion in response to glucose. These results suggest that screening mtDNA mutations in iPSCs from patients

with T2D is an essential step before pancreatic cell differentiation for disease modeling or autologous cell therapy.

Funding Source: The present study was supported by research funds from the National Research Foundation of Korea (NRF-2020M3A9E4036527).
Keywords: Mitochondrial DNA mutation, Pancreatic cell differentiation, Dysfunction of pancreatic cells

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ENDOMETRIAL CANCER ORGANOIDS RETAIN THE HISTOLOGICAL TYPE-SPECIFIC GENOMIC AND EPIGENOMIC FEATURES OF THE ORIGINAL TUMOUR

Semertzidou, Anita - *Metabolism, Digestion & Reproduction, Imperial College London, UK*
Williams, Richard - *Metabolism, Digestion & Reproduction, Imperial College London, UK*
Fernandes, Nadia - *Metabolism, Digestion & Reproduction, Imperial College London, UK*
Brosens, Jan - *Biomedical Sciences, University of Warwick, Coventry, UK*
Kyrgiou, Maria - *Metabolism, Digestion & Reproduction, Imperial College London, UK*

Abstract: Organoids are increasingly being used as complex, multi-dimensional, multi-cell structures resembling entire organs and have now been derived from a variety of tissues. We established endometrial organoid cultures from pipelle biopsies of 11 patients with endometrial cancer (EC) (7 endometrioid, 3 serous, 1 clear cell) and 3 patients with benign conditions. Organoids were grown in Matrigel and medium supplemented with growth factors, Rspondin-1, Noggin, A83-01 and nicotinamide. The genomic and epigenomic features of organoids and parent tissue were compared in pairs and by histological type by targeted gene sequencing and whole-genome DNA methylation profiling. The genetic variations and mutations in seven genes (PTEN, ARID1A, PIK3CA, POLE, CTNNNB1, KRAS, TP53) were largely shared by primary tumours and EC-derived organoids and exhibited histological type-specific characteristics. Similarly, the DNA methylation fingerprint was preserved in cultured endometrial cancer organoids with only few differentially methylated positions (DMPs) compared to tumour tissue. EC epigenetic profiles were distinct to benign endometrial organoids and clustered together according to histotype.

Funding Source: N/A
Keywords: Organoids, Endometrial cancer, genetics

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CANDIDATE SCREEN FOR ENHANCERS OF CAR-T-CELL PERSISTENCE

Nguyen, Kathleen - *Biology, California State University, San Marcos, San Diego, CA, USA*

Abstract: Adoptive cell transfer immunotherapies, including T-cells expressing chimeric antigen receptors (CARs) have shown great success in treating cancer. We have previously developed FT819, a first-of-kind off-the-shelf CAR-T cell therapy derived from a renewable master induced pluripotent stem cell (iPSC) line engineered to uniformly express a CAR targeting CD19 driven by the endogenous T cell receptor (TCR) α promoter at the T-cell receptor α constant (TRAC) locus. Preliminary studies suggested that methods which effectively improved T cell functional persistence conferred greater in vivo tumor control of CAR-T cells. To augment functional persistence of iPSC derived CAR-T (CAR-iT) cells, a group of novel functional modalities, termed T cell enhancers (TCEs), were designed and introduced at the iPSC stage. A previously established iPSC line with a CAR engineered at the TRAC locus was further engineered with these candidate TCEs through lentivirus transduction. The doxycycline-inducible promotor was utilized to allow for control of gene expression by using the Tet-On system. The doxycycline-inducible TCE engineered iPSC lines were differentiated into CD8+ iT cells using our lineage-specification platform where we uniquely generate αβT cells. Serial restimulation assays and tumor killing assays in the presence of doxycycline identified several candidate TCEs that showed increase persistence and tumor killing compared to uninduced iT cell controls. Further studies including in vivo tumor control in mouse xenograft models will reveal the efficacy of these TCEs and the potential of their incorporation into future CAR-iT cell products.

Keywords: CAR-T, iPSC, immunotherapy

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3D VESSEL-ON-CHIP MODEL USING ISOGENIC CADASIL IPSC LINES REVEALS PHENOTYPE SWITCHING IN VASCULAR SMOOTH MUSCLE CELLS (VSMCS)

Vila Cuenca, Marc - *Anatomy and Embryology - Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands*
van den Hil, Francijna - *Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Freund, Christian - *Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Mummery, Christine - *Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Rutten, Julie - *Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands*
Lesnik Oberstein, Saskia - *Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands*
Orlova, Valeria - *Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands*

Abstract: CADASIL is a hereditary small vessel disease caused by NOTCH3 mutations leading to an accumulation and deposition of NOTCH3 protein within the vessel wall. The vascular cells affected in CADASIL are the mural cells (VSMCs and pericytes), which undergo morphological alterations and subsequent degeneration. In this study, we established three clones of isogenic CADASIL and gene-edited control iPSC lines from two CADASIL patients. We first characterized VSMCs derived from these iPSC lines using standard functional assays, such as expression of contractile markers, RNA-seq and high-throughput contraction and intracellular Ca2+ release assays. VSMCs derived from CADASIL iPSC clones (CADASIL iPSC-VSMCs) exhibited abnormal intracellular Ca2+ release and contraction responses upon mechanical and vasoactive stimulation when compared to VSMCs-derived isogenic control iPSC clones. In addition, immunofluorescence staining showed that CADASIL iPSC-VSMCs in 2D adopted a less elongated morphology and reduced levels of contractile markers. Hetero-cellular endothelial cell (EC) and VSMC interaction mediated by Jagged-1 on ECs and NOTCH3 on VSMCs are pivotal for the maturation and function of VSMCs in blood vessels in vivo. We have recently demonstrated that these hetero-cellular interactions can be recapitulated in an iPSC-based engineered

vessel-on-chip model using 3D cell culture chips. Here, 3D vessels-on-chip were generated using CADASIL and isogenic iPSC-derived ECs and VSMCs. In this model, CADASIL and isogenic iPSC-VSMCs acquired positions surrounding the vascular wall, supporting the formation of a microvascular network for up to 7 days. Interestingly, enabling EC-VSMC interaction in 3D, resulted in profound differences between CADASIL and isogenic control iPSC-VSMCs. CADASIL iPSC-VSMCs were larger and more contractile, as confirmed by increased contractile marker SM22 and NOTCH3 expression. Thus, 3D vessel-on-chip model revealed phenotypic differences in CADASIL iPSC-VSMCs that were not recapitulated in 2D. In conclusion, this study demonstrates the utility of the iPSC-based 3D vessel-on-chip system in modelling CADASIL vasculopathy, and enables studies to investigate the effect of NOTCH3 mutations on VSMC behaviour and phenotype.

Funding Source: Novo Nordisk Foundation (NNF21CC0073729). Netherlands Organ-on-Chip Initiative, NWO Gravitation project (024.003.001). University of Pennsylvania OCD with teamCADASIL and cureCADASIL (MDBR2021). ZonMw (VIDI 91717325).
Keywords: Organ-on-chip, CADASIL, vascular smooth muscle cells

TOPIC: CARDIAC

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IN VITRO CARDIAC DIFFERENTIATION OF IPSCS DERIVED FROM PATIENTS WITH A NOVEL DESMIN MUTATION FOR DESMINOPATHY MODELLING

Castañeda, Sheila L. - *Laboratorio de Investigación Aplicado a Neurociencias, Fleni-CONICET, Belén de Escobar, Argentina*
Amin, Guadalupe - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Belli, Carolina B. - *Laboratorio de Genética Hematológica, IMEX-CONICET / Academia Nacional de Medicina, Capital Federal, Argentina*
Smucler, Joaquín - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Möbbs, Alan M. - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Scarafía, María A. - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Waisman, Ariel - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
La Greca, Alejandro - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Sevlever, Gustavo E. - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Miriuka, Santiago G. - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*
Moro, Lucía N. - *Laboratorio de Investigación Aplicado a Neurociencias (LIAN), Fleni-CONICET, Belén de Escobar, Argentina*

Abstract: Desmin (DES) is a type III intermediate filament that attaches organelles and nucleus to contractile apparatus. Aberrant DES isoforms generate protein cytoplasmic aggregates that cause cardiomyopathy. Our main purpose is to model a desminopathy-associated cardiomyopathy in vitro by differentiating human induced pluripotent stem cell (hiPSC) lines to cardiomyocytes (CMs). We generated hiPSC lines after reprogramming peripheral blood mononuclear cells (PBMCs) from one healthy (DES-S) and two diseased (DES-J, DES-Y) siblings that have a non-reported heterozygous mutation in exon 6 of DES gene that generates Glu353 incorporation. After determining normal karyotypes, we detected NANOG, OCT4 and SOX2 expression by RT-qPCR and ability to differentiate to the three germ layers of all hiPSC lines. Once DES-S, DES-J and DES-Y hiPSC lines were validated, we proceeded to differentiate them to CMs using a monolayer protocol. First, we observed that DES-J and DES-Y CMs expressed wild type and mutated DES alleles in similar proportions along cardiac differentiation by RT-qPCR with specific primers. Then, we confirmed that pluripotency genes (NANOG, OCT4) were downregulated from day 3 while mesoderm (MESP1, NKX2.5, TBXT), cardiac (cTnT, CX43, VIM) and desmosomal genes (DSC2, DSG2, DSP, PKG, PKP2) were upregulated at days 3, 7 and 14 within the three cell lines by RT-qPCR. Flow cytometry analysis showed that ~80% of day 3 cells were CD56+ (mesoderm) and ~35% of day 14 cells were cTnT+ for DES-S, DES-J and DES-Y cell lines. CMs MitoTracker incubation revealed higher mitochondrial staining for DES-J and DES-Y respect to DES-S. We also analyzed DES, PKG and PKP2 protein expression in CMs derived from the three hiPSC lines. Lastly, in vitro cardiac contraction was characterized by Contraction Wave software observing higher beating frequency of DES-J and DES-Y CMs compared to DES-S CMs, among other differential parameters. In conclusion, we differentiated DES-S, DES-J and DES-Y validated hiPSC lines to CMs and we observed differences that could be attributed to a cardiomyopathy of a non-reported desminopathy. We expect to deepen in cardiomyopathy modelling and study skeletal muscle desminopathy-associated implications in vitro.

Keywords: DISEASE MODELLING, CARDIAC CONTRACTION, MITOCHONDRIAL AND DESMOSOMAL ARRANGEMENT

TOPIC: EARLY EMBRYO

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REGULATION OF LOW CALCIUM HOMEOSTASIS IN THE EPIDIDYMIS FOR SPERM MATURATION AND MALE FERTILITY

Shum, Winnie - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Zhang, Bao Li - *School of Life Science, Shanghaitech University, Shanghai, China*
Cao, Albert Shang - *School of Life Science, Shanghaitech University, Shanghai, China*
Zhou, Xin - *School of Life Science, Shanghaitech University, Shanghai, China*
Shi, Shuo - *Shanghai Institute for Advanced Immunochemical Studies, Shanghaitech University, Shanghai, China*

Abstract: The epididymis plays an important role in sperm mutations and male fertility, and accumulating evidence indicates that the luminal microenvironment established by epithelial cells is critical for proper epididymal function. Low Ca2+-homeostasis in the epididymis is a critical factor ensuring sperm maturation.

Our research aims to characterise the factors necessary for a favorable epididymal luminal microenvironment and the cellular mechanisms regulating it, particularly as they relate to the low Ca2+ homeostasis. Our previous studies have shown that both the vitamin-D-related TRPV6-TMEP16A channel-coupler and the vitamin-K-dependent GGCX-mediated carboxylation of matrix Gla protein (MGP) regulate Ca2+-homeostasis in the epididymis in a spatially complementary manner. And we found that carboxylated-MGP plays an essential role in promoting Ca2+-dependent protein aggregation. An SNP in the human GGCX gene has also been found to associate with asthenozoospermia. In this study, we provide further evidence to support the notion that luminal matrix Ca2+ functions as a cofactor for carboxylated-MGP scavenging of metabolites in the extracellular microenvironment of mouse epididymis. The MGP-mediated aggregation with a secretory apolipoprotein was altered by changing the in-vitro Ca2+ concentrations. Integrative and bioinformatic analyses suggested the involvement of apolipoprotein receptor LRP2 and multivitamins in the absorption of MGP-bound aggregates in the epididymis. These findings suggest that Ca2+-homeostasis in the epididymis ensures sperm maturation, an important process for male fertility and embryonic development. The Ca2+-dependent MGP scavenging function raises the possibility that the use of multivitamins to modulate Ca2+-homeostasis in epididymis may be leveraged for therapeutic strategies addressing sperm dysfunctions and male reproductive defects.

Funding Source: This work is supported by NNSFC (82071704), SMCST (19140903400) and ShanghaiTech University.
Keywords: Sperm maturation, male fertility, epididymal luminal microenvironment

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A HUMAN EMBRYONIC STEM CELL MODEL OF THE SOX9Y440X CAMPOMELIC DYSPLASIA MUTATION: IMPACT ON NEUROMESODERMAL PROGENITORS

Chu, Ka Chi - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Cheah, Kathryn - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*

Abstract: Campomelic dysplasia (CD) is a rare genetic disease characterized by skeletal abnormalities, congenital heart disease, kidney malformation, hearing impairment, and neurological disorders, which is caused by SOX9 mutation. Among the surviving patients, the SOX9Y440X is one of the most common ones, which gives a truncated SOX9 protein lacking the domain downstream amino acid codon 439. In vitro studies demonstrated that the truncated SOX9 protein contained residual transactivation activity and might act in a dominant negative manner. Yet, understanding about the impact of SOX9Y440X on human embryonic development is limited, as a corresponding disease model is lacking. Here, we established a human embryonic stem cell (hESC) CD model with the aid of CRISPR-Cas9 mutagenesis. In addition, we utilized the hESC CD model to examine the potential impact of the mutation on neuromesodermal progenitors (NMPs), which are the source of majority of the posterior somatic mesoderm and spinal cord. We asked whether the SOX9Y440X mutation interferes with the differentiation and lineage choice of NMPs . We used 10X single-cell transcriptomic profiling to compare the lineage propensity of CD versus wild-type hESCs to differentiate into NMP-like cells. SOX9Y440X hESCs could differentiate into NMPs but a higher proportion of these were biased towards the neural lineage, while a lower proportion contributed to the mesodermal lineage. Analyses of differentially expressed genes revealed altered expression for developmentally crucial genes such as GATA and HES. The hESC CD model will serve as a useful model for understanding of the disease etiology and mechanisms for CD.

Funding Source: HMRF-04153366
Keywords: Campomelic dysplasia, Neuromesodermal progenitors, SOX9

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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MODELING MORE THAN EMBRYONIC DEVELOPMENT: RE-EXAMINING THE GOVERNANCE OF HUMAN EMBRYO AND RELATED RESEARCH

Fabbri, Morris - *Leonard M. Miller School of Medicine and Dr. John T. Macdonald Foundation Department of Human Genetics, John P. Hussman Institute for Human Genomics, University of Miami, , FL, USA*
Munsie, Megan - *Faculty of Medicine, Dentistry and Health Sciences, School of Biomedical Sciences and Melbourne Medical School, Melbourne, Australia*
Ginoza, Margaret - *Leonard M. Miller School of Medicine, University of Miami, FL, USA*
Isasi, Rosario - *Dr. John T. Macdonald Foundation Department of Human Genetics, John P. Hussman Institute for Human Genomics, University of Miami, FL, USA*

Abstract: Following advances in cell reprogramming and culture techniques, researchers can now coax human pluripotent stem cells (hPSC) to imitate the structure and function of the developing human embryo in vitro. While stem cell-based embryo models (SCB-EM) present an alternative to human embryo research, these developments also raise concerns over how such research should be regulated and the adequacy of existing regulatory frameworks and ethics oversight processes. National policies governing stem cell research afford special scrutiny for research involving the human embryo. In the 2021 update to their Guidelines for Stem Cell Research and Clinical Translation, the International Society for Stem Cell Research (ISSCR) posits that oversight bodies specialized to ethically review embryo research should also oversee research on “integrated” SCB-EM, which represent the development of the entire embryo and include relevant embryonic and extra-embryonic structures. In contrast, “non-integrated” models that recapitulate some but not all aspects of the pre-implantation embryo may be reviewed by “existing mandates” which oversee laboratory-based research. While the Guidelines suggest that research should “comply with local law and policy,” it is unclear how national restrictions on embryo research should apply to SCB-EM, which are fundamentally different entities. Using a representative sample of 11 research-intensive countries (United Kingdom, Australia, Israel, Japan, China, Spain, Sweden, Germany, Canada, United States, and France) spanning socio-cultural contexts and policy spectrums, we analyze how evolution of national research policies affects the governance of SCB-EM. We highlight how the overlapping remits of review bodies with different competencies may cause regulatory uncertainty and how mechanisms mandating legislative review could offer a path forward. Finally, we

further compare these policy frameworks against the updated ISSCR Guidelines.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation program iPSpine under grant agreement No. 825925
Keywords: Ethics, Policy, Regulation

TOPIC: EYE AND RETINA

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HYPOXIC PRECONDITIONED MESENCHYMAL STEM CELL DERIVED EXOSOME PROMOTES MITOPHAGY IN THE OPTIC NERVE INJURED BY HYPOXIA THROUGH LONP1/PINK/PARKIN PATHWAY

Lew, Helen - *Department of Ophthalmology, CHA University, Seongnam, Korea*
Park, Mira - *Department of Ophthalmology, CHA University, Seoul, Korea*

Abstract: Stem cell therapy has been widely proposed as an alternative treatment for refractory optic nerve disease. Although mesenchymal stem cells with various tissue regeneration and recovery capabilities are considered as potential cell therapy, they have limitations in the clinical setting. To overcome this problem, we investigated the applicability of extracellular vesicles, exosome, isolated from human placenta-derived mesenchymal stem cells (hPMSCs) as a new treatment. Hpoxia preconditioned hPMSCs (HPPMSCs) were prepared in order to improve nerve recovery function using short-term hypoxic chambers of 2.2% O2 and 5.5% CO2. Then, we isolated exosomes from HPPMSCs with Exoquick. R28 cells, retinal precursor cells, were exposed to CoCl2, and then they were treated with exosome for 24 h. The cell viability was measured by MTT assay and target protein expression and reactive oxygen species (ROS) were analyzed in order to examine mitophagy funtion by exosome. In addition, proteome in each group was analyzed by liquid chromatography/tandem mass spectroscopy. Differential expression proteins (DEPs) were detected by label quantification, and the interaction of proteins were identified. We observed that exosome could restore cell viability and mitochondrial complex activities in R28 cells damaged by CoCl2 exposure. In addition, we identified DEPs in the control group and exosome treatment group related with recovery process ($p < 0.05$). Mitochondria function-related proteins LONP1, PARK7, VDAC1, 2, 3, HSPD1, and HSPA9 were detected in the DEPs list of exosome treatment group. It was confirmed that exosome promoted transcriptions of mitophagy proteins in R28 cells injured by hypoxia, which was not activated in LONP1 knock-downed condition. The pathway could be recapitulated in vivo study. This study presents that LONP1 is a key mediator to promote mitophagy and restore the mitochondria function in the optic nerve injured by hypoxia when treated with preconditioned mesenchymal stem cell derived exsome.

Funding Source: This research was funded by the Ministry of Health & Welfare, Republic of Korea (grant: 2021R1A2C201052311).
Keywords: Optic nerve injury, Exosome, Mitophagy, placenta-derived mesenchymal stem cell, retinal precursor cell, Lonp1, Pink, Parkin

TOPIC: KIDNEY

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ACUTE KIDNEY INJURY MODELLING AND DRUG DEVELOPMENT IN KIDNEY ORGANOIDS

Sander, Veronika - *Department of Molecular Medicine and Pathology, University of Auckland, New Zealand*
Beesley, Pearl - *Department of Molecular Medicine and Pathology, University of Auckland, New Zealand*
Vanichapol, Thitinee - *Department of Molecular Medicine and Pathology, University of Auckland, New Zealand*
Davidson, Alan - *Department of Molecular Medicine and Pathology, University of Auckland, New Zealand*

Abstract: Acute kidney injury (AKI) describes the sudden loss of kidney function, which can be caused by ischemia or the toxic side-effects of clinical drugs. AKI is a common and serious condition for which the underlying pathophysiology is poorly understood, and no approved therapies currently exist. We have previously shown that treating hiPSC-derived kidney organoids with a repeated low-dose regime of the chemotherapeutic drug cisplatin leads to AKI-like phenotypes, including inflammation, DNA damage and cell death. Here, we used magnetic-activated cell sorting to determine the spatial distribution of the cisplatin-induced injury and found that proinflammatory and profibrotic markers, as well as markers for cellular senescence and ER stress are upregulated in the renal tubules of the organoids. Towards developing new therapeutics for AKI, we tested inhibitors of histone deacetylase 8 (HDAC8), which have previously shown promising protective and pro-reparative effects in different AKI models. We found that the HDAC8 inhibitors ameliorated the injurious effects of cisplatin in the organoids. This was also the case in organoid-derived epithelial cells that were cultured in 2D and subjected to cisplatin or hypoxic injury. Using these stem cell-based in vitro tools will help decipher the cellular responses upon AKI and identify cell states, such as senescence and failed tubular cell repair, that could represent therapeutic targets to improve repair.

Keywords: Kidney organoids, Acute kidney injury, HDAC8 inhibitors

TOPIC: MUSCULOSKELETAL

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THE BIG BAG3 THEORY: INSIGHTS FROM HIPSC MODELS FOR BAG3P209L- MYOFIBRILLAR MYOPATHY

Klemm, Kerstin - *Institute of Physiology I, University of Bonn, Germany*
Conklin, Bruce - *Medicine, Pharmacology and Ophthalmology, Gladstone Institutes, San Francisco, CA, USA*
Fleischmann, Bernd - *Institute of Physiology I, University of Bonn, Germany*
Höhfeld, Jörg - *Department of Molecular Cell Biology, University of Bonn, Germany*
Hesse, Michael - *Institute of Physiology I, University of Bonn, Germany*
Judge, Luke - *Clinical Pediatrics, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Patients with an amino acid exchange (p.P209L; c.626C>T) in the BAG3 gene suffer from severe myofibrillar my-

opathy and restrictive cardiomyopathy.The average life expectancy is only about 20 years. BAG3 is a member of the CASA (chaperone-assisted selected autophagy) complex and known to play a key role in the turnover of muscle-proteins. Our goal is to gain a mechanistic understanding of BAG3-associated muscle diseases in order to be able to develop novel therapeutic approaches. To this end, we generated a human induced pluripotent stem cell (iPSC) line harboring the BAG3P209L mutation from a well-characterized iPSC line by gene editing. Furthermore, we are also using an iPSC line derived from a patient with BAG-3P209L-myofibrillar myopathy and a BAG3-/- iPSC line. All these iPSC lines could be successfully differentiated into twitching skeletal muscle cells using an established differentiation protocol, and were phenotypically analysed. Interestingly, both the deletion of the BAG3 gene and the BAG3P209L mutation appear to result in morphological abnormalities, such as the formation of thinner skeletal myotubes and an increase in programmed cell death compared with myotubes derived from control iPSCs. Initial stainings for structural components (e.g. desmin, actin, MF20.) and members of the protein quality control system (e.g. BAG3, HSPB8, LC3B.) also indicate differences. We found that BAG3 protein levels increased in the BAG3P209L patient-derived myotubes compared with controls. Furthermore, we saw significant differences in protein levels of BAG3, p62, and LC3B after the application of physical stress via electrical pulse stimulation. We are currently investigating the mechanisms underlying the disrupted skeletal myocyte phenotype in the disease cell lines by applying cell stress in form of mechanical strain and proteasome inhibition.

Funding Source: Research Training Group 1873, University of Bonn, Germany.
Keywords: Bag3, skeletal muscle cells, iPSC

TOPIC: NEURAL

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NEURON-SUPPORTIVE FUNCTIONS OF IPSC-DERIVED ASTROCYTES GENERATED BY A RAPID DIFFERENTIATION METHOD

Wada, Yuina - *Biomedical Research and Development Department, Biomedical Business Center, RICOH Futures BU, Ricoh Company, Ltd., Kawasaki, Japan*
Yamoto, Rie - *Biomedical Research and Development Department, Biomedical Business Center, RICOH Futures BU, Ricoh Company, Ltd., Kawasaki, Japan*
Ahmad, Muzammil - *Elixirgen Scientific, Inc., Baltimore, MD, USA*
Kilander, Michaela - *Elixirgen Scientific, Inc., Baltimore, MD, USA*
Hosoya, Toshihiko - *Biomedical Research and Development Department, Biomedical Business Center, RICOH Futures BU, Ricoh Company, Ltd., Kawasaki, Japan*

Abstract: The Quick-Tissue™ technology (Elixirgen Scientific, Inc.) is a transcription factor-based method for rapid differentiation of human induced pluripotent stem cells (iPSCs) into desired cell types. The method generates a pure population of astrocytes (Es astrocytes) within 28 days, which is about three times faster than the conventional methods. Astrocytes provide various supportive functions to neurons and contribute to the regulation of the central nervous system. Recent studies have revealed that astrocytes play important roles in the brain function and nervous system diseases. The neuron-astrocyte co-culture system is an in vitro tool that can evaluate the supportive functions of astrocytes and therefore is an attractive test bed for drug screenings, toxicological assays, and disease research. In the present study, we examined whether Es astrocytes provide supportive functions that are similar to that of in vivo astrocytes. We co-cultured neurons with Es astrocytes and characterized their responses to various drugs using calcium imaging. The cells were labeled with the calcium indicator Cal-520 and imaged using the microplate reading system FDSS µCell. The co-cultured neurons responded to antagonists and agonists for receptors of various neurotransmitters as expected. Calcium oscillations of the co-cultured neurons exhibited higher frequency and stronger synchrony compared to that of neuronal cultures without astrocytes, suggesting supportive functions of the astrocytes. The observed supportive functions were robust under a wide range of culture conditions. These results suggest that Es astrocytes generated by the Quick-Tissue™ technology provide neuron-supportive functions that are similar to those of in vivo astrocytes and that they are useful for pharmacological assays of glial functions.

Keywords: astrocytes, neurons, in vitro assay

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MIR203 AS A NOVEL DRIVER FOR MODELLING NEURODEGENERATION-ASSOCIATED CELL DEATH IN VITRO

Patel, Sam - *Neurology, University of California, Los Angeles, CA, USA*
Rexach, Jessica - *Neurology, University of California, Los Angeles, CA, USA*
Geschwind, Daniel - *Neurology, UCLA, Los Angeles, CA, USA*

Abstract: We have identified a novel microRNA (miRNA) module, harbouring a hub driver, miR203, whose overexpression in neurons progressively induced neurodegeneration (ND)-associated phenotypes in neurons and astrocytes. Our next critical question is whether miR203 is present and/or necessary for ND in iPSC models of Tau mutations. We modified R406W, V337M and isogenic MAPT lines with an inducible NGN2 cassette, in order to rapidly generate 2D neuronal cultures in a 96-well format that is compatible with high-content live imaging and automated microscopy analysis. Using this platform, we. (1) assessed miR203 expression in differentiating neurons relative to mutation status, (2) evaluated their sensitization to glutamate-induced cell death and (3) performed miR203 sequestration using our published AAV2/9 viral-based tough decoy (TuD) strategies. Using qPCR, we show >5-fold increase in miR-203 expression in R046W and V337M ISPC lines relative to controls over a relatively short period in NGN2-derived neurons (30 days). Furthermore, we have now shown (i) successful infection (n=6; >50% neurons infected) of days-in-vitro 30 iPSC derived neurons with AAV virus carrying miR-203_TuD sequence to downregulate miR-203 expression. (ii) Whereas, miR-203 expression was significantly increased in R406W MAPT (log fold-change = 11.95 +/- 3.28, p = 0.025, n ≥3) compared to its isogenic control, upon treatment with 8ul AAV-miR-203-TuD, miR203 expression was downregulated to negligible levels (akin to that observed in isogenic control).(iii) AAV-miR-203-TuD also prevented increased cell death downstream of stressor (Glutamate), in R406W mutant neurons (Cell viability w/ and w/out being >60% and < 30%, respectively, p=0.0530, n ≥3). We present miR203 as a novel molecule that could be used to efficiently and consistently model cell death (among many other) aspects of neurodegeneration in a human system. Continued improvement of this platform has now included integration of iPSC derived astrocytes and microglia. These experiments will provide important human functional validation and insight into neuron-glia

interaction in disease-associated neuronal death and promises to accelerate drug development for ND disorders.

Keywords: neurodegeneration, microRNA, tau

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LIS1 RNA-BINDING ORCHESTRATES THE MECHANOSENSITIVE PROPERTIES OF EMBRYONIC STEM CELLS IN AGO2-DEPENDENT AND INDEPENDENT WAYS

Kshirsagar, Aditya - *Departments of Molecular Genetics and Molecular Neuroscience, Weizmann Institute of Science, Rehovot, Israel*
Reiner, Orly - *Departments of Molecular Genetics and Molecular Neuroscience, Weizmann Institute of Science, Rehovot, Israel*

Abstract: LIS1 (Lissencephaly-1) was identified as the first gene involved in a neuronal migration disorder. The absence of the protein results in early embryonic lethality. Yet it has not been established whether Lis1-/- mouse ESCs are viable. We created a specialised medium 5i+LIF that supports Lis1 knockout cell viability using ERT2-Cre. LIS1-GFP OE in Lis1 F/-:ERT2 background versus Lis1 F/-:ERT2 and both treated with tamoxifen were compared to show that LIS1 dosage substantially affected gene expression in mouse ESCs. LIS1 dosage recapitulated a similar effect in human ESCs. We used two human naïve media conditions and four isogenic lines; the control (WIBR3), LIS1+/- lines, LIS1 OE, and a homozygous Lissencephaly mutation in the sixth intron affecting splicing (LIS1-int6*/*) generated with CRISPR/Cas9 genome editing. The enrichment analysis indicated changes in the stemness and differentiation potential, RNA, and the physical properties of the cells, such as mechanotransduction and cell adhesion. LIS1's vital activities are postulated mainly due to its interaction with the molecular motor dynein. But, we demonstrate multiple novel cytoplasmic and nuclear functions of LIS1 in ESCs. The LIS1 protein interacted with numerous RNA-binding proteins, including the Argonaute complex, and we identified AGO-dependent and independent interactions. LIS1 was associated directly with nascent RNA with a preference to introns of highly expressed protein-coding genes. The number of LIS1 binding sites was negatively correlated with intron splicing efficiency, but increased LIS1 showed higher splicing efficiency levels. The expression of miRs located near LIS1 binding sites was also significantly higher than others. LIS1 OE on the background of AGO1-4 KO ESCs partially rescued gene expression and restored the stiffness. Our on-chip platform to grow 3D cortical organoids from mutant human ESCs modelled reduced folding. Extracellular matrix related genes were differentially expressed between control and LIS1+/- organoids at different growth stages. Collectively, our data reveal that LIS1 regulates gene expression and the physical properties together and in parallel to Argonaute proteins. These novel findings change our current perspective on the roles of LIS1 in development.

Keywords: Embryonic stem cells and physical properties, Developmental disorders, Broader significance

743

FIP200 LOSS OF FUNCTION IN HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS RESULTS IN AXONAL PATHOLOGY AND HYPERACTIVITY

Wen, Jianbin - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Bajaj, Thomas - *Department of Psychiatry and Psychotherapy, University Hospital Bonn, Germany*
Brüstle, Oliver - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Braun, Nils - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Gassen, Nils - *Department of Psychiatry and Psychotherapy, University Hospital Bonn, Germany*
Peitz, Michael - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Zellner, Andreas - *Department of Psychiatry and Psychotherapy, University Hospital Bonn, Germany*

Abstract: Genetic studies suggest an association of FIP200 mutations with psychiatric disorders. FIP200 plays important roles in homeostatic processes such as autophagy or signaling pathways such as focal adhesion kinase (FAK) signaling. However, its potential connections to psychiatric disorders and its specific roles in human neurons are not clear. We set out to establish a human-specific model to study functional consequences of neuronal FIP200 deficiency. To this end, we generated two independent sets of isogenic human pluripotent stem cell lines with homozygous FIP-200KO alleles, which were then used for the derivation of glutamatergic neurons via forced expression of NGN2. FIP200KO neurons exhibited pathological axonal swellings and autophagy deficiency with elevated p62 protein levels. Functional studies using multi-electrode arrays showed that FIP200 deficiency results in a hyperactive network. This hyperactivity could be abolished by the glutamate receptor antagonist CNQX, suggesting increased glutamatergic synaptic activation in FIP200KO neurons. Furthermore, cell surface proteomic analysis revealed metabolic dysregulation and abnormal cell adhesion-related processes in FIP200KO neurons. Interestingly, an ULK1/2-specific autophagy inhibitor could recapitulate axonal swellings and hyperactivity in wild-type neurons, whereas inhibition of FAK signaling was able to normalize the hyperactivity of FIP200KO neurons. These data suggest that both, impaired autophagy and disinhibition of FAK contribute to the hyperactivity of FIP200KO neuronal networks, whereas pathological axonal swellings are mainly induced by autophagy deficiency. Considering that schizophrenia patients were reported to exhibit hippocampal and cortical hyperactivity, our observations might contribute to the dissection of FIP200-associated cellular and molecular pathomechanisms contributing to neuropsychiatric disease.

Funding Source: German Federal Ministry of Education and Research grant 01ZX1314A, the Horizon 2020 Research & Innovation Action (grant agreement No. 667301), China Scholarship Council, and the University of Bonn International Studies Program.
Keywords: forward programming, neuronal hyperactivity, psychiatric disorders

745

EXPLOITING OLIGODENDROCYTES-ENRICHED 3D SPHEROIDS TO DISSECT GLOBOID CELL LEUKODYSTROPHY PATHOGENESIS

Mangiameli, Elisabeth - *SR-Tiget, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milano, Italy*
Freschi, Marta - *SR-Tiget, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy*
Gritti, Angela - *SR-Tiget, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy*

Abstract: Globoid Cell Leukodystrophy (GLD) is a lysosomal storage disorder due to genetic deficiency of galactosylceramidase (GALC), a key enzyme in sphingolipid metabolism. GLD manifest with rapid and relentless demyelination/neurodegeneration affecting central and peripheral nervous system. To date, the pathogenic mechanisms leading from the primary defect to overt tissue damage are not fully understood. Our work on human patient-specific iPSC (hiPSC)-derived neural progenitor cells (NPCs) and mixed neuronal/glial 2D cultures showed that a timely regulated GALC expression is critical during neural commitment and highlighted a defective neuronal and oligodendroglial differentiation in GLD patients' cells. Still, the 2D culture conditions did not support the full maturation of hiPSC-derived neurons and oligodendrocytes (OL), the latter cell type being primarily affected by GALC deficiency and consequent accumulation of the toxic metabolite psychosine during myelin turnover. Thus, we envisage that 3D OL-enriched spheroids allowing extensive maturation of both neuronal and glial cell populations may represent a more suitable experimental model to investigate the early neurodevelopmental defects linked to the GLD pathology as well as the impact of GALC deficiency during OL maturation and myelination. Here, we generated 3D OL-enriched spheroids from available GLD hiPSC clones and from newly established GALC knock-out (KO) and knock-in (KI) hiPSC clones obtained by CRISPR-Cas9-mediated gene editing of a healthy donor (HD)-derived hiPSC clone. We characterized the spheroids by means of molecular and immunophenotypical analysis. Our preliminary results suggest that: i) OL-enriched 3D spheroid can be generated with comparable efficiency from HD, GLD and GALC isogenic hiPSCs; ii) Mature MBP+ OL and MAP2+ neurons are present in both HD and GLD organoids. We are currently evaluating the kinetics of neuronal and glial differentiation/maturation, the presence of compactly myelinated axons, as well as the appearance of GLD pathological hallmarks (e.g. senescence, apoptosis, lysosomal defects) using biochemical and molecular techniques coupled with -OMICS and advanced imaging approaches.

Keywords: Globoid cell leukodystrophy, Brain organoids, oligodendrocytes

TOPIC: PANCREAS

747

DE NOVE MODELING OF PANCREATIC CANCER USING HUMAN EMBRYONIC STEM CELL DERIVED PANCREATIC ORGANOIDS REVEALS DUAL PATHWAY FOR TUMORIGENESIS IN PDAC

Zhang, Xian - *Development Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Wong, Wilfred - *Weill Cornell Medical College, Weill Cornell Graduate School of Medical Sciences, New York, NY, USA*

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is projected to be the second leading cause of cancer related death in U.S. The lack of faithful human cell-based models that recapitulate PDAC initiation and progression thwarts the effort to develop screening and therapeutic methods. We have established a novel human pluripotent stem cell-derived gene editing-amiable pancreatic organoid (PO) culture system to reconstitute the genetic features of PDAC development. Expression of oncogenic KRAS and combinatorial deletion of classical tumor suppressors TP53, CDKN2A with or without SMAD4 in the POs recapitulated molecular hallmarks of malignant transition in vitro and histopathology of early and late stages of PDAC upon orthotopic transplantation. Expression of human specific PDAC marker CEACAM5/6 and patient PDAC validated disease progression marker TRIM29 and PTGES in in vitro cultured POs representing early and late stage of PDAC revealed therapeutic targets and biomarker discovery potential of the model. Transcriptome and chromatin accessibility profiling have detected ectopic activation of AP-1 motif enriched chromatin region and suppression of endoderm differentiation features in PDAC-mimicking POs, suggesting essential signal pathways in the acquisition of malignancy in pancreatic epithelium. Pharmacological inhibition of ERK signaling as well as genetic perturbation of PDAC specific AP-1 factors in PDAC-mimicking POs partially reverse the malignant transformation feature. These findings validate the pluripotent stem cell based PDAC model a valuable opportunity for mechanism study of PDAC development and a unique platform for therapeutic drug discovery.

Keywords: pancreatic ductal adenocarcinoma, pancreatic organoid, CRISPR

TOPIC: PLURIPOTENT STEM CELLS

749

NOVEL INSIGHTS FROM METABOLOMIC ANALYSIS OF HUMAN NEURONS DERIVED FROM PATIENTS WITH MECP2-RELATED SYNDROMES

Osenberg, Sivan *Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA-*
Sheppard, Jennifer - *Department of Pediatrics, Baylor College Of Medicine, Houston, TX, USA*
Bajic, Aleksandar - *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA*
Khalil, Saleh - *Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA*
Mahat, Aditya - *Pediatrics, Baylor College of Medicine, Houston, TX, USA*
Suter, Bernhard - *Departments of Neurology and Pediatrics, Baylor College Of Medicine, Houston, TX, USA*

Pehlivan, Davut - *Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA*
Li, Feng - *Department of Pathology & Immunology, Baylor College of Medicine, Houston, TX, USA*
Maletic-Savatic, Mirjana - *Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA*

Abstract: The brain is exquisitely sensitive to gene dosage: the correct dosage is important for cellular homeostasis, while altered dosage (loss or gain) leads to pathology. Altered dosage of the X-linked gene MeCP2 results in clinically similar neurodevelopmental disorders associated with autism spectrum disorder, learning deficits, epilepsy, and impaired motor function: too much of it leads to MeCP2 Duplication Syndrome (MDS) seen mostly in boys, and too little of it causes Rett syndrome seen mostly in girls. Despite the well-established genetic basis of the MeCP2-related disorders, the affected cellular processes underlying these disorders remain poorly understood and disease-modifying treatments are still lacking. Consequently, treatment options for patients are currently limited and centered on symptom relief. Recent studies indicated that there are altered carbohydrates in the cerebrospinal fluid, increased leptin, pyruvate, and lactate in serum, and aberrant cholesterol metabolism in Rett mouse models and patients, suggesting that Rett syndrome might be a metabolic disease. We modeled MDS and Rett syndrome using induced pluripotent stem cells (iPSCs) derived from molecularly confirmed male MDS and male Rett (MRL, to minimize sex effect) patient-specific fibroblasts and healthy controls. We examined the metabolome of iPSC-derived neurons from 8 MDS, 6 MRL and 2 controls and found significant differences mainly involving polyamine, purine, cholesterol-derived and mitochondrial pathways. Interestingly, we detected significantly higher levels of some neurotransmitters (such as nicotinamide and glutamate) in MDS neurons than in MRL and control neurons. These metabolomic differences in patient-specific induced neurons might uncover in vitro signatures that distinguish gene dosage effects (MDS vs MRL) and provide further understanding of MECP2-related syndromes. Ultimately, these data may help identify novel potential therapeutic targets or prognostic marker(s) for disease progression, toxicity, and therapeutic efficacy.

Keywords: MECP2, Rett, MECP2 duplication

751

COACTIVATION OF GSK3B AND IGF-1 ATTENUATES AMYOTROPHIC LATERAL SCLEROSIS NERVE FIBER CYTOPATHIES IN SOD1 MUTANT PATIENT-DERIVED MOTOR NEURONS

Chang, Chia-Yu - *Neuroscience Center, Hualien Tzu Chi Hospital, Hualien City, Taiwan*
Ting, Hsiao-Chien - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
Yang, Hui-I - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
Harn, Horng-Jyh - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
Chiu, Ing-Ming - *Institute of Cellular and System Medicine, National Health Research Institutes, Miaoli, Taiwan*
Su, Hong-Lin - *Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan*
Li, Xiang - *Waisman Center, University of Wisconsin, Madison, WI, USA*
Chen, Mei-Fang - *Department of Medical Research, Hualien Tzu Chi Hospital, Hualien, Taiwan*
Ho, Tsung-Jung - *Department of Chinese Medicine, Hualien Tzu Chi Hospital, Hualien, Taiwan*
Liu, Ching-Ann - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
Tsai, Yung-Jen - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
Chiou, Tzyy-Wen - *Department of Life Science, National Dong Hwa University, Hualien, Taiwan*
Lin, Shinn-Zong - *Bioinnovation Center, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive nervous system disease that causes motor neuron (MN) degeneration and results in patient death within a few years. To recapitulate the cytopathies of ALS patients' MNs, SOD1G85R mutant and corrected SOD1G85G isogenic-induced pluripotent stem cell (iPSC) lines were established. Two SOD1 mutant ALS (SOD1G85R and SOD1D90A), two SOD1 mutant corrected (SOD1G85G and SOD1D90D), and one sporadic ALS iPSC lines were directed toward MNs. After receiving ~90% purity for MNs, we first demonstrated that SOD1G85R mutant ALS MNs recapitulated ALS-specific nerve fiber aggregates, similar to SOD1D90A ALS MNs in a previous study. Moreover, we found that both SOD1 mutant MNs showed ALS-specific neurite degenerations and neurotransmitter-induced calcium hyperresponsiveness. In a small compound test using these MNs, we demonstrated that gastrodin, a major ingredient of Gastrodia elata, showed therapeutic effects that decreased nerve fiber cytopathies and reverse neurotransmitter-induced hyperresponsiveness. The therapeutic effects of gastrodin applied not only to SOD1 ALS MNs but also to sporadic ALS MNs and SOD1G93A ALS mice. Moreover, we found that coactivation of the GSK3β and IGF-1 pathways was a mechanism involved in the therapeutic effects of gastrodin. Thus, the coordination of compounds that activate these two mechanisms could reduce nerve fiber cytopathies in SOD1 ALS MNs. Interestingly, the therapeutic role of GSK3β activation on SOD1 ALS MNs in the present study was in contrast to the role previously reported in research using cell line- or transgenic animal-based models. In conclusion, we iden-tified in vitro ALS-specific nerve fiber and neurofunctional markers in MNs, which will be useful for drug screening, and we used an iPSC-based model to reveal novel therapeutic mechanisms (including GSK3β and IGF-1 activation) that may serve as potential targets for ALS therapy.

Funding Source: Buddhist Tzu Chi Medical Foundation, Hualien Tzu Chi Hospital , Ministry of Science and Technology (MOST 107-2314-B-303-001-, MOST 108-2314-B-303-009-, MOST 109-2314-B-303-023-, MOST 110-2314-B-303-025-), Taiwan.
Keywords: amyotrophic lateral sclerosis (ALS), induced pluripotent stem cell (iPSC), motor neuron (MN)

POSTER SESSION I: ODD

6:30 PM – 7:30 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: EARLY EMBRYO

503

DEVELOPING A HIGH-CONTENT CELL LINEAGE TRACING METHOD FOR MOUSE EMBRYOGENESIS

Masuyama, Nanami - *Faculty of Applied Science and Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*
Kijima, Yusuke - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*
Mori, Hideto - *Graduate School of Media and Governance, Keio University, Kanagawa, Japan*
Nishizono, Hirofumi - *Medical Research Institute, Kanazawa Medical University, Kahoku, Japan*
Negishi, Mikiko - *Research Center for Advanced Science and Technology, The University of Tokyo, Japan*
Kagieva, Madina - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*
Matsumoto, Shoma - *Research Center for Animal Life Science, Shiga University of Medical Science, Ootsu, Japan*
Okamura, Eiichi - *Research Center for Animal Life Science, Shiga University of Medical Science, Ootsu, Japan*
Adel, Arman - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*
Sakata, Rina - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*
Toyoshima, Hiromi - *Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Japan*
Kakoi, Kentaro - *Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Japan*
Ishiguro, Soh - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, Canada*
Tanimoto, Yoko - *Laboratory Animal Resource Center, University of Tsukuba, Japan*
Masui, Osamu - *Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan*
Seki, Motoaki - *Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Japan*
Nishida, Keiji - *Graduate School of Science, Technology and Innovation, Kobe University, Kobe, Japan*
Koseki, Haruhiko - *Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan*
Aburatani, Hiroyuki - *Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Japan*
Ema, Masatsugu - *Research Center for Animal Life Science, Shiga University of Medical Science, Ootsu, Japan*
Mizuno, Seiya - *Laboratory Animal Resource Center, University of Tsukuba, Japan*
Yachie, Nozomu - *Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*

Abstract: The mammalian development starts from a single fertilized egg that proliferates to form complex structures in the body. Several approaches have been reported to trace cell lineages of metazoan species, whereby chromosome-embedded DNA barcodes are continuously mutated by CRISPR-Cas9 genome editing and inherited from mother to daughter cells. In such a system, the mutation patterns of the descendant barcodes allow the reconstruction of the developmental lineage, akin to evolutionary phylogeny estimation. However, no previous methods have achieved lineage tracing of large systems like whole-body animal development at the resolution of a single cell. The recordable information content of the previous barcoding systems has been limited, mainly because DNA barcodes are short, and Cas9 induces a limited pattern of mutations mainly by deletions and is toxic to cells. Furthermore, all previous methods have only been validated in part by comparing the reconstructed lineages with single-cell RNA sequencing (scRNA-seq)-based cell states or histological annotations, but not comprehensively by orthogonal methods. In this conference, we will present a novel high-content lineage tracing approach “Barclock” that we have been developing in the last several years. The Barclock system employs CRISPR base editing for the massively parallel recording of cell lineage information into multiple hundreds of gRNA targeting sequences and scRNA-seq for reading of the recorded memory information. We are developing mice lines based on this system to decipher the whole-body cell lineages of embryogenesis at an unprecedented scale.

Keywords: Lineage tracing, DNA barcode, Embryogenesis

TOPIC: EPITHELIAL_GUT

505

A FULLY FUNCTIONAL CONTRACTILE GASTROINTESTINAL ORGANOID SYSTEM GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS TO DEVELOP A HIGH THROUGHPUT SYSTEM TO DETECT NEWLY EMERGING PATHOGENS

Michkov, Alexander V. - *Biomedical Science Division, NeyroblastGX LLC, Murrieta, CA, USA*
Lutz, Holly - *Department of Pediatrics and Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA*
Gilbert, Jack - *Department of Pediatrics and Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA*
Begum, Aynun - *Biomedical Science Division, NeyroblastGX LLC, Murrieta, CA, USA*

Abstract: The increasing global population brings the added risk of newly emerging pathogens due to their resistance to current antibiotics or vaccines. In addition, people or warfighters now travel from one place to another to complete their assigned mission and non-mission activities or leisure. Thus people are regularly exposed to new environments with new pathogens. Increased travel permits the rapid transmission of pathogens from place to place. COVID-19 pandemic is a remarkable example of how rapidly pathogens can evolve and infect people. NeyroblastGX LLC (NGL) developed “hOPSenseTM,” a human induced pluripotent stem cell (hiPSC)-derived 3D gastrointestinal organoid (GIO) system compatible with high-throughput (HT) electroconductive sensing arrays for detecting physiologically relevant pathogen

responses. NGL electroconductive hOPSense pathogen sensing device built on a hiPSC-derived 3D human GIO with contractile ability replicates pathogen attack in vivo. Our GIO system identifies many pathogen responses (>20 pathogens) within an hour with higher sensitivity and specificity. We treated our GIO with healthy microbiota, which maintained the microbiome similar to in vivo conditions, and differentiated pathogens and non-pathogens with a reproducible electrical signal. We also performed shotgun metagenomics, which confirmed that our GIO could significantly differentiate the microbiome versus pathobiome as similar to the electrical signal response of hOPSense. Therefore, NGL's GIO system can be used to determine civilian or war fighter's microbiome after exposure to new pathogens, disease, and endotoxins in the intestine rapidly and on a robust scale. This HT-scale GIO electroconductive system "hOPSense" is a cost-effective human intestinal model and can fulfill the demand for the pathogen, drug, or toxicity screening through electrical signals that mimic the in vivo physiological responses and replace the high-costs of animal and human clinical trials in the future.

Funding Source: This work is supported by Defense Advanced Research Agency (DARPA).
Keywords: Human 3D gastrointestinal organoid from HiPSC, Electroconductive pathogen sensing arrays, shotgun metagenomics

TOPIC: HEMATOPOIETIC SYSTEM

507

LINEAGE-SPECIFIC DOWNREGULATION OF LRF IN HEMATOPOIETIC STEM CELLS FOR THE INDUCTION OF FETAL HEMOGLOBIN IN ERYTHROID CELLS

Devaraju, Nivedhitha - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Balasubramanian, Poonkuzhali - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Chambayil, Karthik - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Murugesan, Mohankumar - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Nakamura, Yukio - *Cell Engineering Division, Riken, Ibaraki, Japan*
Pai, Ashwin - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Rajendiran, Vignesh - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Srivastava, Alok - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Thangavel, Saravanabhavan - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Velayudhan, Shaji - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Venkatesan, Vigneswaran - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Bagchi, Abhirup - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*

Abstract: Human β globin gene expression undergo developmental switch from embryonic to fetal to adult-expression. Any mutations in the adult globin gene results in inherited monogenic blood disorders such as β-thalassemia and sickle cell disease (SCD). Several clinical data across the world have reported that the survival of certain β-thalassemia or SCD patients are due to the hereditary persistence of fetal globin expression in adult stage. Thus, reactivation of fetal haemoglobin in adult stage to overcome the severity of β- globin abnormality by modifying the expression of γ-globin repressors has become a favourable approach. ZBTB7A codes for a ZBTB transcription factor called LRF, binds to DNA through C-terminal C2H2-type zinc fingers and recruits transcriptional repressor to repress the target gene. Reports strongly suggest that disruption of LRF, de-represses gamma globin and results in elevated HbF production. However, the role of LRF as a regulatory repressor is also observed in HSC maintenance, T cell fate decision , adipogenesis and myeloid maturation. Therefore, we aimed to achieve lineage-specific downregulation of LRF at optimal repression, sufficient to reactivate HbF without compensating its role other compartments of haematopoiesis in HSPCs. To do this, we first screened the different shRNAs against ZBTB7ALRF in HUDEP -2 erythroid cell line and selected the shRNA that showed both efficient knockdown and fetal globin reactivation. The downregulation of LRF in healthy donor HSPCs was carried out using the selected shRNA at VCN~ 1 which showed ~30 % elevation in HbF HPLC chain analysis. Increase in VCN to further increase HbF elevation resulted in delayed or minimal defect in erythroid maturation compared control. Further, on performing preliminary transplantation experiments in immunocompromised NSG mouse model to study the effect of lineage specific LRF knockdown in HSPCs we observed comparable engraftment between the LRF downregulated HSPCs and the control cells. Ex-vivo erythropoiesis of the bone marrow cell showed 40% CD71+GFP expressing erythroid cells with significant elevation in fetal globin. These observations suggests that lineage specific minimal knockdown of LRF elevates HbF at significant levels but the observed on erythroid cell maturation defect has to be examined further.

Keywords: LRF/ZBTB7A, fetal globin, lineage specific downregulation

TOPIC: KIDNEY

509

INTERPRETING GEOMETRIC RULES OF EARLY KIDNEY FORMATION FOR SYNTHETIC MORPHOGENESIS

Hughes, Alex - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Viola, John - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Porter, Catherine - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Liu, Jiageng - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Gupta, Ananya - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Alibekova Long, Mariia (Masha) - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Prahl, Louis - *Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*

Abstract: The intricate branching of the kidney's urinary collecting duct tree occurs in response to tightly choreographed cues passed between ureteric epithelial tubules and surrounding nephron progenitors during development. The tips of this epithelial network are then the engines that drive formation of the

200,000-2 million nephron filters present in adult kidneys. However, the engineering principles governing the remarkable development of this tubule network are not well understood. Thus, there is massive potential to study and apply new engineering rules that would shed light on the wide variability in nephron number between individuals, which correlates with adult disease, and to build large enough kidney tissues for functional replacement. We first cast tubule network formation in the developing mouse kidney as a packing problem using organ explants and physical models. Our data indicate that developing kidneys visit only a narrow range of possible tubule packing solutions and predict two classes of packing defects that can be found in published literature. Furthermore, they suggest that internal, radially oriented forces on tubules are required to avoid these defects and for tubule trees to resolve into vertically packed arrays. We validate this by perturbing live kidney explants and matching outcomes to our physical models. Our data suggest two new geometric principles for synthetic morphogenesis efforts - anisotropic stresses in nephron progenitor domains associated with tip packing, and ureteric tubule tension. We therefore outline two engineering strategies based on these principles. In one example, we synthetically guide ureteric tubule networks by drawing parallels between programmed strain in micropatterned tissues and the ureteric branching program. We reconstitute these mechanics in tissue scaffolds that progressively encode 3D extracellular microenvironments by programmed shape change. By integrating microfluidic cell and matrix patterning, kinematic models, and tubule engineering, we describe a vision for encoding long-range patterning in next-generation kidney organoids.

Funding Source: This work was supported by NIH F32 DK126385 to LSP, NIH T32HD083185 to JMV, NIH MIRA R35GM133380 to AJH, NSF GRFP to CMP, and NSF CAREER award to AJH.
Keywords: Kidney development, Synthetic morphogenesis, Stem cell mechanics

TOPIC: MUSCULOSKELETAL

511

DISSECTING 3D GENE EXPRESSION PATTERNS IN SKELETAL MUSCLE

Martinez Mir, Clara - *Anatomy and Embryology, Leiden University Medical Center and The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden node, Leiden, Netherlands*
Boers, Ruben - *Developmental Biology, Erasmus MC and Oncode Institute, Rotterdam, Netherlands*
Alemany, Anna - *Anatomy and Embryology, Leiden University Medical Center and The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden node, Leiden, Netherlands*
Gribnau, Joost - *Developmental Biology, Erasmus MC and Oncode Institute, Rotterdam, Netherlands*
Sage, Fanny - *Anatomy and Embryology, Leiden University Medical Center and The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden node, Leiden, Netherlands*
Geijsen, Niels - *Anatomy and Embryology, Leiden University Medical Center and The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden node, Leiden, Netherlands*

Abstract: Skeletal muscles are complex. They consist of multinucleated myofibers, blood vessels, nerve fibers, and connective tissue. During embryonic and fetal development myoblasts fuse to form multinucleated myofibers that vary in size, shape and arrangement. The number of myonuclei is highly variable among the different muscles, with tibialis anterior (TA) myofibers containing up to 1000 while extensor digitorum longus (EDL) fibres having between 200 and 300 myonuclei. It is well-known that within myofibers, RNA and protein are organized in different specialized compartments, and that particular localization patterns vary depending on topography. Single-nucleus transcriptomics analysis has revealed distinctive myonuclear populations, suggesting complex gene expression regulation; however, tissue dissociation methods used in these analyses result in complete loss of spatial information. We've used a spatial transcriptomics and epigenomics approach, combining traditional cryosectioning of full TA muscles in mice, followed by transcriptomic (RNAseq) and methylome (MeDseq) sequencing of sequential sections. We have identified regionalized expression and methylation patterns along the anterior-posterior axis of these TAs, related to spatial-restricted myofiber functionality.

Funding Source: This project was supported, in part, by a grant from the Novo Nordisk Foundation (NNF21CC0073729).
Keywords: Heterogeneity, Compartmentalization, Spatial multiomics

TOPIC: NEURAL

513

HIGH-DENSITY RECORDINGS FOR LONG-TERM ELECTROPHYSIOLOGY IN DEVELOPING BRAIN ORGANOIDS

Sevetson, Jess - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Voitiuk, Kate - *Bioinformatics, University of California, Santa Cruz, CA, USA*
Robbins, Ash - *Electrical and Computer Engineering, University of California, Santa Cruz, CA, USA*
Parks, David - *Computer Science, University of California, Santa Cruz, CA, USA*
Elliott, Matt - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Spaeth, Alex - *Computer Engineering, University of California, Santa Cruz, CA, USA*
Paz Flores, Claudia - *Genomics Institute, University of California, Santa Cruz, CA, USA*
Teodorescu, Mircea - *Electrical and Computer Engineering, University of California, Santa Cruz, CA, USA*
Haussler, David - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*
Salama, Sofie - *Biomolecular Engineering, University of California, Santa Cruz, CA, USA*

Abstract: Human stem cell derived cerebral cortical organoids are three-dimensional biological models of the human brain that recapitulate aspects of early human development. These organoids offer unprecedented access to developing human neural tissues, and recent work demonstrates the existence of coordinated activity. Applications of cerebral organoids include human evolution, modeling neurodegenerative disease, congenital brain malformations, major psychiatric disorders, and personalized medicine. Although organoids are physiologically relevant models of the developing brain, widespread adoption of these models is hindered by technical difficulties in measuring complex neural network activity. Current methods involve slicing the organoid and severing the developing network in half. We pres-

ent a longitudinal electrophysiology platform using high-density multielectrode arrays, enabling us to record over several days without severing network connections within the organoids. This platform allows us to observe network dynamics as they naturally emerge. The proposed system addresses a significant limitation with in vitro neuroscience and will help realize the full potential of organoid models. These fundamental improvements will facilitate measuring the contributions of human-specific genes and variants on the development of functional neural networks using the organoid model.

Funding Source: K12GM139185 (JLS), NIH/NIMH R01MH120295 (SRS), Schmidt Futures Foundation SF 857 (D.H.) and NSF 2034037 (M.T.), D.H. is an HHMI Investigator,
Keywords: organoid, neurodevelopment, electrophysiology

TOPIC: NT - GENERAL

515

LIGATION-ASSISTED HOMOLOGOUS RECOMBINATION ENABLES PRECISE GENOME EDITING BY DEPLOYING BOTH MMEJ AND HDR

Geijsen, Niels - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Shang, Peng - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: CRISPR/Cas12a is a single effector nuclease that, like CRISPR/Cas9, has been harnessed for genome editing based on its ability to generate targeted DNA double strand breaks (DSBs). Unlike the blunt-end DSB generated by Cas9, Cas12a generates sticky-end DSB that could potentially facilitate precise genome editing, but this unique feature has thus far not been much deployed for this purpose. Here we present 'LAHR' (Ligation-Assisted Homologous Recombination), a novel and efficient method for precise genome modification. LAHR utilized a combination of HDR and microhomology-mediated end joining (MMEJ) to insert a double-stranded DNA template with matching 5' overhang at the Cas12a target site. We demonstrate that LAHR can be applied both for specific base substitutions as well as insertions. As such, the LAHR genome editing strategy extended the repertoire of precise genome editing approaches and provided a widened understanding of the type and role of DNA repair mechanisms involved in genome editing.

Funding Source: The work was supported, in part, by a grant from the China Scholarship Council, the H2020 iPSpine project and RegMedXB.
Keywords: CRISPR-Cas12a, Precise Genome Editing, MMEJ and HDR

517

ILLUMINATING DYNAMIC STRUCTURAL STATES IN HIPSCS THROUGH ENDOGENOUS GENE TAGGING

Fuqua, Margaret - *Stem Cells and Gene Editing, Allen Institute, Seattle, WA, USA*
Roberts, Brock - *Stem Cells and Gene Editing, Allen Institute, Seattle, WA, USA*
Gunawardane, Ruwanthi - *Stem Cells and Gene Editing, Allen Institute, Seattle, WA, USA*

Abstract: The Allen Institute for Cell Science has created a collection of endogenously tagged Human Induced Pluripotent Stem Cell (hiPSC) lines to illuminate cell organization. To date, the Allen Cell Collection consists of 52 single- or dual-edited lines that have undergone extensive quality control testing to ensure genomic, cell biological, and stem cell integrity. We have tagged many commonly recognized membrane-bound and membrane-less cellular organelles, signaling complexes, phase transition markers, transcription factors, and cardiomyocyte-specific structural markers. Two of our most recently released lines CTCF-mEGFP and PCNA-mEGFP for dynamic visualization of distinctly associated chromatin domains and replication foci, respectively. We have also released a cell line with endogenously tagged G3BP1-mEGFP to label stress granules. We recently initiated endogenous tagging of several cadherins, including E-cadherin, N-Cadherin and VE-cadherin. This work highlights our gene-editing and quality control workflows for mono- and biallelic editing of expressed or silent genes that are expressed specifically during differentiation and function as reporters of cellular state. Furthermore, we share efforts to improve our endogenous gene tagging workflow utilizing Adeno-Associated Virus (AAV) to deliver donor DNAs for gene tagging at multiple loci, enabling us to generate gene edited cell lines with greater efficiency. Our cell lines, the donors 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 Tagging, Genome Editing, Live Cell Imaging

519

DEVELOPMENT OF FGF2 HOMOLOGS FOR APPLICATIONS IN HUMAN STEM CELL CULTURE AND THE CULTIVATED MEAT INDUSTRY

Rees, Steven D. - *Executive, Defined Bioscience, Inc., San Diego, CA, USA*
Schlagal, Caitlin - *Research and Development, Defined Bioscience, Inc., San Diego, CA, USA*
Karpiak, Jerome - *Research and Development, Defined Bioscience, Inc., San Diego, CA, USA*

Abstract: Fibroblast growth factor 2 (FGF2 or basic fibroblast growth factor, bFGF) is a multifunctional secreted signaling protein found in a variety of adult and embryonic cell types. A member of the FGF1 subfamily of canonical FGFs, FGF2 engages heparin/heparan sulfate (HS) as a co-factor for triggering dimerization of FGF receptors (FGFRs) 1-3, effecting tyrosine kinase phosphorylation and downstream signaling across pathways critical to growth, differentiation, migration, and survival (including RAS/MAPK, PI3K-AKT, PLCy-PKC, STAT, and others). Initially discovered in the growth of chicken periosteal fibroblasts in the 1930s, FGFs have been instrumental in the maintenance and differentiation of stem cells, particularly in serum-free medium formulations. FGF2 has been further improved in the past decade with mutations derived from molecular modeling and empirical validation, aiming to promote higher function and thermostability. These methods have largely been focused on human FGF2 for use in conspecific cell culture. The growing cultivated meat industry will require high-functioning, low-cost, and species-specific growth factors for large-scale manufacturing. To explore evolution-driven changes in FGF2 across species relevant to the cultivated meat industry, we produced homologs of FGF2 found in a range of mammalian, avian, and piscine species including zebrafish. These proteins, when validated in standard growth as-

says, highlight the versatility of growth factors produced from alternative species for use in human- and animal-derived stem cell applications. Subsequent efforts, complemented by a 3D crystal structure of hyper-stable human FGF2-G3 generated in-house, will explore inherent and mutation-driven hyper-stability of these factors for use in improving growth factor stability, shelf-life, and function.

Funding Source: This work was supported by Good Food Institute grant REES042021.
Keywords: Protein engineering, Growth factors, Cultivated meat

TOPIC: PLURIPOTENT STEM CELLS

521

ROBUST WORKFLOWS FOR THE EXPANSION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN AGGREGATES IN SUSPENSION CULTURE

Jervis, Eric - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Walsh, Kylan - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Gill, Jaspreet - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
McManus, Sarah - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Lin, Leon - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Walasek, Marta - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Chang, Wing - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Wognum, Albertus - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Corporate Affairs, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research & Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: STEMCELL's portfolio of TeSR™ 3D-based media products have been developed for robust and scalable suspension culture of human pluripotent stem cells (hPSCs) in aggregates. However, the field has been challenged by the lack of methods that can reproducibly scale hPSCs cultures without prior adaptation of cells to the higher shear levels imposed in stirred suspension. A critical balance exists between the agitation rate to maintain aggregates in suspension and the generation of shear by agitation. Agitation methods were tested for their ability to maintain aggregate suspensions and cell growth rates. Experiments were conducted with 6 different cell lines (3 PSC and 3 iPSC) in which cells were serially expanded in suspension cultures up to 500 mL. Aggregates were passaged non-enzymatically by dissociation using Gentle Cell Dissociation Reagent and filter-based trituration. The only system that gave reproducible growth across cell lines had a low-shear Vertical-Wheel® impeller design. With this workflow, hPSCs underwent a greater than 1.5- to 1.9-fold expansion per day (cell line dependent) with > 85% viability, > 90% expression of OCT4 and TRA-1-60, the capacity to differentiate to three germ layers, and a normal karyotype. To verify the utility of this workflow, 2 hPSC lines were further differentiated into polyploid megakaryocytes (MKs) in 3D suspension cultures. Differentiation used established 2D protocols with a 12-day endothelial-to-hematopoietic transition phase, and a 5-day progenitor-to-mature MK stage. At the end of the protocol, 45 - 75% of cells expressed CD41a, 25 - 65% of the cells co-expressed CD41a and CD42b, and 10 - 60 CD41a+CD42b+ cells were generated per seeded hPSC (n = 9). The DNA ploidy profile of the CD41a+CD42b+ cells generated showed 26% and 9% of cells had 4N and 8N+ DNA ploidy, respectively. The combination of TeSR 3D workflows and low-shear bioreactors provides a robust system suitable for the expansion of a wide range of hPSC lines.

Keywords: Cell Manufacturing, Suspension Culture of PSCs, Differentiation in Suspension Culture

523

LIVE HIPSC SORTING FOR PLURIPOTENCY AND HIGH VIABILITY USING CELL SURFACE MARKERS SSEA-4 AND TRA-1-60-R

Eribez, Korina D. - *Applications, NanoCellect Biomedical, Inc., San Diego, CA, USA*

Abstract: The culture, handling, and sorting of induced pluripotent stem cells (iPSCs) require special care that can be both time consuming and laborious in order to achieve reliable results. Maintaining the naïve state and pluripotency in culture is reliant on regulating variables such as nutrient composition, temperature, and other developmental cues. Although maintaining a homogeneous stem cell culture is possible, researchers greatly benefit from other tools to succeed and reduce laborious maintenance. Microfluidic cell sorting is essential to the success of gently sorting homogeneous stem cells and eliminating unwanted cells. Here we demonstrate how the dual laser WOLF G2 Cell Sorter was imperative to identify and sort naïve stem cell populations. The WOLF G2 accurately identified and enriched hiPSCs that were labeled with two cell surface markers SSEA-4 (Stage Specific Embryo Antigen 4) and TRA-1-60-R (Tumor-related Antigen-1-60 [R]), that are widely used to label undifferentiated stem cells. Using these surface markers proved successful by starting with a 69.6% double-positive sample and purifying to 92.8%. In addition, presort cells stained with SYTOX™ Green Ready Flow™ Reagent dead cell stain displayed roughly 6% dead cells and was improved to > 99% viable cells post-sort. This high purity and viability of the stem cells was also affirmed by successful seeding and adherence of the sorted iPSCs into a new 6-well plate. Day 5 colony morphology from re-seeded wells exhibited healthy characteristics such as distinct borders, well defined edges, and a large nucleus to cytoplasm ratio. NanoCellect's microfluidic sorting technology together with defined and animal-free growth medium that enhances retention of naivety and pluripotency, enables researchers to generate consistent high-quality results. Overall, the gentle sorting and positive identification of such a demanding and delicate cell type has demonstrated the high utility of the WOLF G2.

Keywords: Microfluidic sorting, SSEA-4, TRA-1-60-R

525

ANALYTICAL PERFORMANCE OF THE ICS-DIGITAL(TM) PSC TEST

Miermont, Agnès - *Research and Development, Stem Genomics, Montpellier Cedex 5, France*
Assou, Said - *R&D, IRMB, Montpellier Cedex 5, France*
Bouaud, Marie - *Technical Department, Stem Genomics, Montpellier Cedex 5, France*

De Vos, John - *R&D, IRMB, Montpellier Cedex 5, France*
Hauser, Elena - *Technical Department, Stem Genomics, Montpellier Cedex 5, France*

Abstract: The iCS-digitalTM PSC 24-probe test is a multiplexed digital PCR assay for the identification of the most recurrent Copy Number Variants (CNVs) occurring in cultured human Pluripotent Stem Cells (hPSC)1-2. This test is based on digital PCR technology which enables sequence-specific detection and absolute quantification of nucleic acids. Use of the iCS-digitalTM PSC test for routine testing purposes requires validation to ensure proper quality control of hPSC genome integrity. Herein, we present the results of an analytical performance study of the iCS-digitalTM PSC test.

Keywords: human pluripotent stem cells PSCs cells in culture hPSC genomic integrity, Copy Number Variants (CNVs), digital PCR

527

3D STEM CELL CULTURE, ORGANOIDS AND WOUND TREATMENT WITH NANOFIBRILLAR CELLULOSE

Kiuru, Tony - *Biomedicals, UPM Kymmene Corporation, Helsinki, Finland*
Mikkonen, Piia - *Biomedicals, UPM Kymmene Corporation, Helsinki, Finland*
Sheard, Jonathan - *Biomedicals, UPM Kymmene Corporation, Helsinki, Finland*
Ståhlberg, Roosa - *Biomedicals, UPM Kymmene Corporation, Helsinki, Finland*

Abstract: Three-dimensional (3D) cell culture models are needed to provide better biologic relevance and functionality compared to traditional 2D in vitro cultures. Nanofibrillar cellulose (NFC) hydrogel (GrowDex®) is a highly biocompatible material shown to provide an effective support matrix for culturing various cell types in 3D. As an animal-free matrix, NFC enables possibilities also for clinical applications. (A) Pluripotent stem cells were embedded in NFC hydrogel and cultured up to 26 days. Pluripotency was analysed with OCT4 and SSEA-4 marker expression, in vitro EB-mediated differentiation, and teratoma assay. Cells proliferated in NFC without feeder cells, formed spheroids with 100-200 µm diameter, and the cells retained their pluripotency without changes in karyotypes. (B) Renal organoids can mimic the structure and function of in vivo kidneys. Organoids were cultured from primary mouse embryonic kidney metanephric mesenchymal cells followed by chemical induction to undergo nephrogenesis. Cells were embedded in NFC which reduced the stress-induced affects during the nephrogenesis process. This allowed the organoids to grow in conditions mimicking better the physiological environment. (C) Wound healing is a complex and continuous process which involves a variety of cells, soluble factors, and extracellular matrices. The potential of NFC for wound treatment was studied as hydrogel-based dressing and hydrogels. In addition, potential of NFC as a cell scaffold for human adipose-derived mesenchymal stromal cells (hASCs) was studied. Clinically, NFC wound dressing provided efficient wound healing at skin graft donor sites, and as a hydrogel it did not affect wound closure rate in vivo or altered a normal healing process. Foreign-body reaction was also not observed. hASCs cultured on top of NFC dressing presented to maintain their undifferentiated state, immunological properties and high cell viability. These findings offer a good platform to continue the development of the cell-based wound treatment. Nanofibrillar cellulose is a biocompatible material that offers a well-defined 3D culture matrix for various cell types in vitro and enables opportunities also in regenerative medicine applications. Authors would like to thank Yan-Ru Lou, Ulla Saarela, and Jasmi Snirvi for performing the experiments.

Keywords: pluripotent stem cells, kidney organoids, wound care

529

A SYNTHETIC CYTOKINE RECEPTOR PLATFORM FOR PRODUCING CYTOTOXIC INNATE LYMPHOCYTES AS OFF-THE-SHELF CANCER THERAPEUTICS

Rowland, Teisha - *Engineered Allogeneic Therapies, Umoja Biopharma, Seattle, WA, USA*
Vereide, David - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
O'Hara, Samantha - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
Koning, Ryan - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
Hoffmann, Michele - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
Yingst, Ashley - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
Nicolai, Chris - *Research, Umoja Biopharma, Seattle, WA, USA*
Pankau, Mark - *Vector Biology, Umoja Biopharma, Seattle, WA, USA*
Mittelsteadt, Kristen - *Discovery, Umoja Biopharma, Seattle, WA, USA*
Michels, Kathryn - *Immunology, Umoja Biopharma, Seattle, WA, USA*
Shin, Seungjin - *Vector Biology, Umoja Biopharma, Seattle, WA, USA*
Beitz, Laurie - *Discovery, Umoja Biopharma, Seattle, WA, USA*
Ryu, Byoung - *Vector Biology, Umoja Biopharma, Seattle, WA, USA*
Crisman, Ryan - *Technical Operations, Umoja Biopharma, Seattle, WA, USA*
Scharenberg, Andrew - *Umoja Biopharma, Umoja Biopharma, Seattle, WA, USA*
Garbe, Christopher - *Engineered Allogeneic Therapies, Umoja Biopharma, Boulder, CO, USA*
Larson, Ryan - *Immunology, Umoja Biopharma, Seattle, WA, USA*

Abstract: While induced pluripotent stem cells (iPSCs) hold promise as a renewable, modifiable, and scalable source of material for cancer immunotherapies, current immune cell differentiation approaches are complex and require the addition of cytokines, and in some cases feeder cells. Here, we present a platform for producing effector immune cells in the absence of cytokines by genetically modifying iPSC derivatives to express a synthetic cytokine receptor, the rapamycin-activated cytokine receptor (RACR). RACR is activated via addition of a synthetic small molecule ligand, driving differentiation and expansion of lymphocytes we term RACR-induced Cytotoxic Innate Lymphoid (iCILs) cells. Starting from the undifferentiated iPSC state, over the course of RACR-driven iCIL differentiation and feeder-free expansion, we observe an approximate 3000-fold cell expansion. Because RACR induces a signal analogous to IL-2 and IL-15 which are both associated with immune cell proliferation, iCILs possess a selective growth advantage, resulting in a highly pure iCIL population. Functionally, iCILs mediate cytotoxic activity against target tumor cells and secrete IFNγ and TNFα mediated by innate receptors and engagement of a chimeric antigen receptor (CAR). Importantly, RACR can be employed both in a manufacturing setting

to drive iCIL production and in vivo to enable selective expansion and persistence of iCILs. We are currently testing iCILs in humanized mouse models and in small scale-up settings to further evaluate their unique properties and in vivo anti-tumor activity. Taken together, these data demonstrate the capacity of our platform to use rationally-designed receptors to produce synthetic lymphocytes with significant promise as "off-the-shelf" cancer immunotherapy cell products. In particular, this approach has the potential to improve the manufacturing process of iPSC-based allogeneic cell therapies by increasing process control and product consistency, while decreasing cost and complexity. We believe our platform will be amenable to the controlled, cost-effective production of iCILs and other synthetic immune cell types for off-the-shelf therapeutic applications targeting hematological and solid tumors.

Keywords: cancer, immunotherapy, chimeric antigen receptor (CAR)

TOPIC: GERMLINE

753

APPLICATION OF RNA VIRAL VECTOR WITH GUANINE RIBOSWITCH TO PRIMORDIAL GERM CELL-LIKE CELL INDUCTION

Kim, Narae - *Nucleic Acid Chemistry and Engineering Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Japan*
Yokobayashi, Yohei - *Nucleic Acid Chemistry and Engineering Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Japan*

Abstract: RNA viral vectors equipped with a riboswitch are promising tools for controlling transgene expression by a small molecule to induce cell differentiation. Because the RNA viral vector does not go through a DNA intermediate, it is particularly useful for germ cell induction from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) avoiding heritable genomic alterations. Previously, we found a vesicular stomatitis virus (VSV) mutant which efficiently replicates in mouse ESCs. In this study, the VSV vector carrying Prdm14 gene was equipped with guanine-responsive riboswitch (off-switch) to allow chemical control of the transgene expression. Mouse ESCs were infected with the VSV-Prdm14 vector and cultured in a mouse ESC medium under drug selection. To induce primordial germ cell differentiation, the cells were cultured in a medium supplemented with Activin A and basic FGF for 2 days which induces epiblast stem cell-like cells (EpiLCs) from naive state ESCs cultured with PD0325901, CHIR99021, and LIF. Gene expression profile analyzed by qPCR showed significant downregulation of Nanog and Klf4 from naive ESCs to EpiLCs and upregulation of Fgf5 and Otx2 under the transgene suppression condition in the presence of guanine. The induced EpiLCs were then dissociated and transferred to a microwell plate to make embryoid bodies (EBs) for primordial germ cell-like cell induction. Although the endogenous gene expression of Prdm1, Tfap2c and Mvh showed a similar gene expression pattern with or without guanine at day 2 and day 4 of EB, Stella/Dppa3 was upregulated without guanine at day 2 of EB with exogenous Prdm14 expression. Thus, the VSV-Prdm14 vector could be replicated in ESCs while suppressing transgene expression in the presence of guanine, and induction of Prdm14 expression upon removal of guanine during EB formation could induce expression of genes that regulate germ cell specification.

Funding Source: The research was funded by Okinawa Institute of Science and Technology Graduate University and Japan Society for the Promotion of Science (JSPS) KAKENHI grants 20K15669 and 19H02855.
Keywords: riboswitch, embryonic stem cells, primordial germ cells

TOPIC: NEURAL

755

LONG-TERM ADHERENCE OF HUMAN BRAIN CELLS IN VITRO IS ENHANCED BY NEW POLYMER COATINGS

Bardy, Cedric - *South Australian Health and Medical Research Institute (SAHMRI), Flinders University, Adelaide, Australia*
Milky, Bridget - *SAHMRI, Flinders University, Adelaide, Australia*
Zabolocki, Michael - *SAHMRI, Flinders University, Adelaide, Australia*
Al-Bataineh, Sameer - *TekCyte Pty Ltd., Adelaide, Australia*
Van den Hurk, Mark - *SAHMRI, Adelaide, Australia*
Greenberg, Zarina - *SAHMRI, Adelaide, Australia*
Turner, Lucy - *SAHMRI, Adelaide, Australia*
Mazzachi, Paris - *SAHMRI, Flinders University, Adelaide, Australia*
Williams, Amber - *SAHMRI, Flinders University, Adelaide, Australia*
Lleperuma, Imanthi - *SAHMRI, Flinders University, Adelaide, Australia*
Adams, Robert - *SAHMRI, Flinders University, Adelaide, Australia*
Stringer, Brett - *SAHMRI, Flinders University, Adelaide, Australia*
Ormsby, Rebecca – *Flinders Health and Medical Research Institute, Flinders University, Adelaide, Australia*
Poonnoose, Santosh - *Flinders Health and Medical Research Institute, Flinders University, Adelaide, Australia*
Smith, Louise - *TekCyte Pty Ltd., Adelaide, Australia*
Krasowska, Marta - *STEM, University of South Australia, Adelaide, Australia*
Whittle, Jason - *STEM, University of South Australia, Adelaide, Australia*
Simula, Antonio - *TekCyte Pty Ltd., Adelaide, Australia*

Abstract: Advances in cellular reprogramming have radically increased the use of patient-derived cells for neurological research in vitro. However, adherence of human neurons on tissue cultureware is unreliable over the extended periods required for electrophysiological maturation. Adherence issues are particularly prominent for transferable glass coverslips, hindering imaging and electrophysiological assays. Here we assessed thin-film plasma polymer treatments, polymeric factors and extracellular matrix coatings for extending adherence of human neuronal cultures on glass. We find positively charged, amine-based plasma polymers improve adherence of a range of human brain cells. Diaminopropane (DAP) treatment with laminin-based coating optimally supported long-term maturation of fundamental ion channel properties and synaptic activity of human neurons. As proof of concept, we demonstrated that DAP-treated glass is ideal for live imaging, patch-clamping and optogenetics. DAP-treated glass surface reduces the technical variability of human neuronal models and enhances electrophysiological maturation, allowing more reliable

discoveries of treatments for neurological and psychiatric disorders.

Keywords: iPSC, Neurons, electrophysiology

TOPIC: NT - GENERAL

757

STARBRIGHT DYE ANTIBODY CONJUGATES: NEW TOOLBOX FOR YOUR STEM CELL RESEARCH

Huang, Leon - *Life Science Group, Bio-Rad Laboratories, Hercules, CA, USA*
Li, Lei - *Life Science Group, Bio-Rad Laboratories, Hercules, CA, USA*
Blundell, Michael - *Life Science Group, Bio-Rad Laboratories, Oxfordshire, UK*

Abstract: In this study, the excitation and emission spectra of all StarBright Dyes were checked by spectrophotometer and evaluated by spillover on flow cytometry. Moreover, SBV515 was conjugated to anti-CD8 antibody and used for cell staining. The spillover value was analyzed and compared to the control BV510. Much less spillover was noticed on multiple channels (e.g., 405-670/30 nm, 405-615/24 nm). To test the stability of StarBright Dyes, short and long-term ambient light exposure (4 and14 days) were performed using the anti-CD14-SBV610 conjugate. The percentage of positive population was identical by staining with 4 and 14 days ambient light exposed StarBright Dye conjugates, compared to regular staining with conjugate stored in the dark. Meanwhile, methanol (90% and 100%) treatment was applied to test the StarBright Dye stability under fixation and permeabilization. After methanol treatment, fluorescent intensity in the anti-CD4-PE control group (stain index) was significantly decreased by up to 10 fold, but not in the anti-CD4-SBY575 group. Measured by spillover on flow cytometry, the spectral signatures of PE, but not SBY575, were dramatically changed after treatment with methanol. Furthermore, different staining buffers and fixation compatibility were evaluated on the StarBright Dye conjugates. Human peripheral blood was stained with anti-CD4-SBV515 in FACS buffer (1XDPBS, 1% BSA), Bio-Rad Stain Buffer, or Brilliant Stain buffer (BD Biosciences). Similar fluorescent intensity and percentage of positive cell population were observed in all conditions. For the fixation compatibility test, cells were fixed in either Bio-Rad Fixation Buffer or 2% paraformaldehyde before data acquisition, following cell surface staining. There were no differences in the fluorescent intensity and percentage of positive cell population in fixation and fresh staining groups. Finally, an eight-color flow cytometry panel combining StarBright Dye antibody conjugates with traditional fluorophore-conjugated antibodies was built to enable the detection of hematopoietic stem cells (HSCs) with B and T cell lineages in human peripheral mononuclear cells using the Bio-Rad ZE5 Cell Analyzer.

Keywords: StarBright Dye, Antibody, Flow cytometry

TOPIC: PANCREAS

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LIGHT-STIMULATED CALCIUM ION INFLUX IN PANCREATIC ISLET-LIKE ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Choi, Jieun - *Biological Sciences, KAIST, Daejeon, Korea*
Shin, Eunji - *Biological Sciences, KAIST, Daejeon, Korea*
Lee, Jinsu - *Biological Sciences, KAIST, Daejeon, Korea*
Heo, Won do - *Biological Sciences, KAIST, Daejeon, Korea*
Han, Yong-mahn - *Biological Sciences, KAIST, Daejeon, Korea*

Abstract: Optogenetics has several advantages such as non-invasiveness, spatio-temporality, and reversibility in regulation of cellular function. Here, we report an optogenetic regulatory system for manipulating intracellular calcium ion level ([Ca2+]i) in hPSC-derived pancreatic islet-like organoids (PIOs). monSTIM1 (monster-opto-Stromal interaction molecule 1) is a synthetic protein designed to spatiotemporally modulate the [Ca2+]i in various biological model systems. monSTIM1 transgene was integrated at AAVS1 loci of both alleles in human embryonic stem cells (hESCs) by CRISPR-Cas9-mediated genome editing. Generated homozygous monSTIM1+/+-hESCs exhibited light-responsiveness of [Ca2+]i transient via the endogenous CRAC channel. Moreover, monSTIM1+/+-hESCs successfully differentiated to PIOs, and β-cells in monSTIM1+/+-PIOs also showed a reversible, repetitive [Ca2+]i transient dynamics upon light stimulation. Our results demonstrate human cellular model for optogenetic control of the [Ca2+]i in pancreatic endocrine β-cells, which can be potentially applied for optogenetic control of the insulin secretion.

Keywords: Pancreatic islet-like organoid, Optogenetics, Calcium ion influx

POSTER SESSION I: ODD

6:30 PM – 7:30 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

603

THE SPONTANEOUS MULTIDIRECTIONAL CELL DIFFERENTIATION IN ADSC-DERIVED SPHEROIDS

Gorkun, Anastasiya - *Department of Molecular and Cellular Pathophysiology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Winston Salem, NC, USA*
Kolokoltsova, Tamara - *Department of Molecular and Cellular Pathophysiology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia*
Kosheleva, Nastasia - *World-Class Research Center "Digital Biodesign and Personalized Healthcare", Sechenov First Moscow State Medical University, Moscow, Russia*
Nikishin, Denis - *Embryonic Physiology, Koltzov Institute of Developmental Biology of Russian Academy of Sciences,*

Moscow, Russia
Revokatova, Darya - *Department of Molecular and Cellular Pathophysiology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia*
Saburina, Irina - *Department of Molecular and Cellular Pathophysiology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia*
Shpichka, Anastasia - *Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, Moscow, Russia*
Timashev, Peter - *Sechenov Biomedical Science & Technology Park, Sechenov First Moscow State Medical University, Moscow, Russia*
Zurina, Irina - *Department of Molecular and Cellular Pathophysiology, Research Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia*

Abstract: Spheroid cell culture showed an advantage before 2D cultures in the increasing cell differentiation potential and plasticity. This study shows that 3D culture promotes spontaneous multidirectional differentiation of adipose-derived stromal cells (ADSCs). The study was conducted using human ADSCs isolated from an adipose stromal-vascular fraction. Cells were confirmed for expression multipotential cell main markers as CD29, CD44, CD73, CD90, CD105 and for not expressing any markers associated with osteogenesis, vascular cells, adipogenesis, and chondrogenesis in 2D culture. Experimental groups included a group of intact spheroids and groups with different combinations of inducing factors. Spheroids were characterized using SEM, IHC staining, real-time PCR, Western Blot, and angiogenesis assay at a different time of 3D cultivation. SEM images confirmed that all spheroids had the standard morphology with surface epithelial-like cells and central stromal cells surrounded by an extracellular matrix. Complex analysis of expression and synthesis of key factors (Osteopontin, Osteocalcin, CD31, Flk1) indicated that ADSC acquire a capacity for spontaneous osteogenesis and partial endothelial-like differentiation when cultured in the form of spheroids. At the same time, standard osteogenic and angiogenic exogenic factors were not capable of influencing cell differentiation within spheroids in a persistent and time-dependent manner but affected further morphogenesis. Thus, spheroids from different groups formed CD34+ tubule-like structures in fibrin gel, and the addition of osteogenic factors led to the formation of a more branched and less structured net of tubules. Additionally, intact ADSC-derived spheroids showed spontaneous expression of Adiponectin (adipocyte marker) and Collagen II (chondrocyte marker). PCR analysis showed the increase of expression of pluripotent genes (Oct4, Nanog, and C-Myc) during the first hours of spheroidogenesis. To sum up, the current study showed that ADSC spheroids are capable of spontaneous differentiation in different directions that can be connected with de novo obtained pluripotency of cells. These results open a wide range of new approaches in both fundamental and applied research in the area of tissue development and regeneration.

Funding Source: This work was supported by the Russian Federation President grant MK-3776.2019.4, The Special Federal Programme of the Russian Federation Government, Research Project No. FGFU-2022-0009, and RFBR, project number N° 20-02-00712.
Keywords: Adipose-derived stromal cells, Spheroid, Spontaneous differentiation

TOPIC: CARDIAC

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DEVELOPMENT OF MULTI-CELLULAR VASCULARIZED CARDIAC TISSUE USING 3D BIOPRINTING AND IPSC-CMS

Hwang, Boeun - *Biomedical Engineering, Emory University, Decatur, GA, USA*
Jin, Linqi - *Biomedical Engineering, Emory University, Atlanta, GA, USA*
Tomov, Martin - *Biomedical Engineering, Emory University, Atlanta, GA, USA*
Serpooshan, Vahid - *Biomedical Engineering, Emory University, Atlanta, GA, USA*

Abstract: Developing engineered heart tissues has been a focus in cardiovascular and regenerative medicine. Recently, the fabrication of cardiac tissue constructs has been accelerated by advances in stem cell technologies which enable large-scale cultures of cardiomyocytes (CMs). Engineered cardiac tissues have been utilized as platforms for drug development and as therapeutic/regenerative devices. However, challenges in engineered heart tissues, such as limited tissue size and lack of complexity, remain to be overcome. In this study, we present a new state-of-art cardiac tissue manufacturing which integrates human induced pluripotent stem cell (hiPSC) and 3D bioprinting technologies, to recapitulate the multi-cellular architecture and complex functional vasculature of myocardial tissue. Pre-vascularized cardiac tissue was fabricated by 3D bioprinting a sacrificial vascular tree between two layers of hiPSC-derived CMs (hiPSC-CMs) encapsulated in the bioink consisting of gelatin methacrylate (gelMA), gelatin, and fibrinogen. This cellular bioink was first cast in a custom-designed mold. Then a multi-scale vascular tree was bioprinted using the sacrificial pluronic F-127, onto which another layer of hiPSC-CM bioink was cast. The assembly of the cellular construct was crosslinked with UV and thrombin solution, subsequently. Pluronic was removed to generate hollow channels within the construct. Human umbilical vein endothelial cells (HUVECs) were next seeded within the open vasculature to create a fully endothelialized cardiac tissue. The gelMA-gelatin-fibrin bioink provided a favorable environment for hiPSC-CM survival and function. CMs encapsulated in the bioink formed cell-to-cell connections and exhibited synchronous contractions. This bioprinted tissue was further functionalized by the incorporation of HUVECs. The HUVECs seeded in the channels of the cardiac tissue emulate blood vessels and enable nutrient transport throughout the tissue. The uniform layer of endothelium sustained its structure under flow. The hiPSC-derived vascularized human cardiac tissues, created using multimaterial 3D bioprinting, can not only be used as an in vitro platform to study cardiovascular diseases but also as a therapeutic device, i.e., cardiac patch, to regenerate the damaged myocardium.

Funding Source: American Heart Association (906506) supported B.H.
Keywords: Tissue Engineering, 3D Bioprinting, Cardiovascular Regeneration

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

607

IDENTIFYING THE SITES OF DNA DAMAGE RELATED TO CELLULAR SENESCENCE BY A MODIFIED CRISPR SCREEN

Ito, Satoshi T. - *Graduate School of Frontier Sciences, The University of Tokyo, Japan*
Ohta, Sho - *The Institute of Medical Science, The University of Tokyo, Japan*
Yamada, Yasuhiro - *The Institute of Medical Science, The University of Tokyo, Japan*

Abstract: Cellular senescence is a persistent proliferative arrest induced by a variety of stresses. It is suggested that cells become senescence in organismal aging, tissue repair, and wound healing. Accumulating senescent cells may promote age-related phenotypes and diseases. One of the most critical stresses is a DNA double-strand break (DSB). When it occurs, damage-sensor proteins such as ATM recognize the stress and activate p53. Then, p53 upregulates effectors and arrests the cell cycle. Finally, cells are going to become senescent. However, DSBs cause not only senescence but other cellular responses, such as continuing proliferation and apoptosis. Although previous studies using the conventional method, ultraviolet lights or DNA damaging reagents, cleared the signal pathway from DNA damages, how and which factors related to DSBs determine the cellular response is largely unknown. To figure out the function of the DSBs which induce cellular senescence, we created a "CRISPR-dCas9-scFokI" system, a novel experimental platform based on CRISPR-Cas9. A catalytically dead Cas9 is fused with tandem endonuclease domains, FokI, to separate DNA recognition and cleavage sites in this platform. Thus, this approach probably allows repeated DSBs at a specific locus. As of today, we have confirmed that this system generates DSBs in NIH3T3, and our data suggest that CRISPR-dCas9-scFokI is less mutagenetic than CRISPR-Cas9. Furthermore, we hypothesized that a pooled CRISPR screen detects the genome sites related to cellular senescence. We are currently setting up a screening method for mouse fibroblasts. In the future, we are planning to generate an aging mouse model using CRISPR-dCas9-scFokI and the results of the screen and analyze the potential of tissue repair and wound healing in the mice. Here, we would like to discuss recent progress on this project.

Keywords: Cellular senescence, a pooled CRISPR screen, DNA damage

TOPIC: EPITHELIAL_GUT

609

REWIRING MACROPHAGE NRG1 SIGNALLING INTO A SYNTHETIC SYSTEM TO CONTROL GUT BEHAVIOUR

Berrocal, Miguel A. - *Anatomy and Physiology, University of Melbourne, Carlton, Australia*
Clark, Michael - *Anatomy and Physiology, The University of Melbourne, Australia*
De Paoli, Ricardo - *Anatomy and Physiology, The University of Melbourne, Australia*
Heath, Daniel - *Engineering and IT, The University of Melbourne, Australia*
Wells, Christine - *Anatomy and Physiology, The University of Melbourne, Australia*

Abstract: Neuregulin-1 (NRG1) is a pleiotropic growth factor that signals via EGF receptors. NRG1 belongs to a family of transmembrane proteins that are regulated through a complex combination of differential transcriptional start sites, alternative exon splicing, and post-transcriptional cleavage of propeptides. This complexity coincides with the role of NRG1 isoforms in many fundamental developmental and tissue regulatory processes. For instance, NRG1 is essential for healthy organ development, cell maturation and metabolism, with defined isoforms playing a role in the heart, skeletal muscle or neurons. NRG1 has been reported as an important growth factor that triggers cell division in the gut stem cell niche, where macrophage NRG1 expression regulates tissue architecture of the gut from its crypts. We hypothesize that NRG1 is one of the missing links in in vitro developmental protocols of multicellular organoids. We have identified multiple NRG1 isoforms expressed in human macrophages, which include a myeloid specific isoform that was previously uncharacterised. Applying principles of synthetic biology and genetic engineering to stem-cell derived macrophages and gut epithelium, we aim to design a system in which we can control NRG1 isoform specificity, and track NRG1 signaling. Our aim is to develop NRG-1 controlled synthetic circuits that not only illuminate the cross talk between macrophages and other tissues like the gut, but also demonstrate the application of human stem cells as a chassis for orthogonal circuits with predictable and programmable outcomes.

Keywords: Synthetic Biology, Neuregulin-1, Macrophage engineering

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DIFFERENTIATION TO FETAL-LIKE STATUS BY IN VIVO PARTIAL REPROGRAMMING PROMOTES INTESTINAL REGENERATION

Kim, Jumee - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kim, Somi - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Jo, Beom-Ki - *College of Pharmacy, Seoul National University, Seoul, Korea*
Oh, Ji-Young - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kim, Keun-Tae - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kim, Jong Kyoung - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Koo, Bon-Kyoung - *Center for Genome Engineering, Institute for Basic Science, Daejeon, Korea*
Cha, Hyuk-jin - *College of Pharmacy, Seoul National University, Seoul, Korea*

Abstract: There is emerging evidence that dedifferentiation with fetal-like gene signature or formation of revival stem cells (revSCs) occurs in the damaged intestine for repair. Here, we demonstrated that, by driving forced dedifferentiation of intestinal epithelium using Yamanaka factors (e.g., Oct4, Sox2, Klf4 and c-Myc: OSKM), partial reprogramming promoted intestinal repair upon ionizing radiation (IR) injury. Single cell RNA sequencing revealed that partial reprogramming triggered plasticity of entire intestinal epithelium to produce a distinct population with fetal-like gene signature, highly resembling revSCs, and contribute to epithelial regeneration. Intestinal organoid model, recapitulating the de-

differentiation and fetal-like transition by partial reprogramming, demonstrated that activation of YAP was required for the acquisition of fetal-like characteristics. These studies, therefore, suggest that transient dedifferentiation by OSKM upon damaged tissue would be a potential therapeutic approach for improving the limited regeneration capacity.

Keywords: In vivo partial reprogramming, Intestinal regeneration, Dedifferentiation

TOPIC: EYE AND RETINA

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MICROCARRIER-BASED CULTURE OF HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL PIGMENT EPITHELIUM

Faynus, Mohamed - *Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Pennington, Britney - *Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Bailey, Jeffrey - *Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Clegg, Dennis - *Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*

Abstract: Dry age-related macular degeneration (AMD) is estimated to impact nearly 300 million individuals globally by 2040. While no treatment options are currently available, multiple clinical trials are currently underway investigating retinal pigment epithelial cells (RPE) derived from human pluripotent stem cells (hPSCs) as a cellular replacement therapeutic. It has been estimated that a production capacity of >1010 RPE cells per year would be required to treat the afflicted population, but current manufacturing protocols are labor intensive, time consuming, and typically limited to scales of 108-109 RPE cells per batch. Microcarrier technology has enabled high-density propagation of many adherent mammalian cell types via monolayer culture on tiny spherical surfaces suspended in medium. While microcarriers have been widely adopted for therapeutic protein production, few studies have explored microcarrier-based culture of RPE cells. Here we provide an approach to the growth, maturation, and cryopreservation of hPSC-RPE cells on several types of commercially available microcarriers. We demonstrate that hPSC-RPE adhere to microcarriers coated with collagen, vitronectin or matrigel, and that the cells mature in vitro to exhibit distinct epithelial cell morphology and pigmentation. Microcarrier-grown hPSC-RPE (MC-RPE) express RPE signature genes including BEST1, RPE65, TYRP1, and PMEL, secrete the neurotrophic factor PEDF, and demonstrate phagocytosis of photoreceptor outer segments. Furthermore, cryopreserved MC-RPE retain high viability, expression of identity marker genes, and RPE function post-thaw. The capacity to support hPSC-RPE cultures using microcarriers enables the efficient large-scale production of therapeutic RPE to meet the treatment demands of a large AMD patient population.

Keywords: Microcarrier, Retinal Pigment Epithelium, Cryopreservation

TOPIC: HEMATOPOIETIC SYSTEM

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THE ENDOSOMAL ADAPTOR PROTEIN MYCT1 CONTROLS ENVIRONMENTAL SENSING IN HUMAN HSCS

Aguade Gorgorio, Julia - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Jami-alahmadi, Yasaman - *Department of Biological Chemistry, University of California, Los Angeles, CA, USA*
Kardouh, Maya - *University of California, Los Angeles, CA, USA*
Fares, Iman - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Calvanese, Vincenzo - *Laboratory for Molecular Cell Biology, University College London, UK*
Johnson, Haley - *Molecular Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Magnusson, Mattias - *Division of Molecular Medicine and Gene Therapy, Lund University, Lund, Sweden*
Shin, Juliana - *Cedars Sinai, Los Angeles, CA, USA*
Goodridge, Helen - *Cedars Sinai, Los Angeles, CA, USA*
Wohlschlegel, James - *Department of Biological Chemistry, University of California, Los Angeles, CA, USA*
Mikkola, Hanna - *Molecular Cell and Developmental Biology, University of California, Los Angeles, CA, USA*

Abstract: Hematopoietic stem cells (HSC) integrate diverse environmental cues to maintain the balance between quiescence, self-renewal and differentiation throughout life. However, the molecular programs governing HSC stemness become dysregulated during culture, compromising HSC self-renewal and engraftment ability. Despite recent advances in optimizing culture conditions for human HSC, our ability to expand or modify functional human HSCs in culture for therapeutic use is still limited. To understand the molecular programs responsible for maintaining human HSC function, we analyzed multiple gene expression data sets of cultured and uncultured human HSPCs from developmental and postnatal hematopoietic tissues. We discovered that MYCT1 (Myc target 1) gene is selectively expressed in undifferentiated HSPCs and endothelial cells (EC) but becomes drastically downregulated during culture, concomitantly with loss of engraftment potential. Knockdown (KD) experiments revealed that MYCT1 is critical for human HSC expansion and engraftment. Using subcellular fractionation, immunofluorescence, and immunoprecipitation coupled with high-sensitivity mass spectrometry we discovered that MYCT1 is a membrane-associated protein localized in endosomes, where it interacts with vesicle trafficking components and signaling receptors with critical functions in HSC biology. MYCT1 KD in human ECs and HSPCs led to hyperactivation of endocytosis, a crucial regulatory step determining the responsiveness to extracellular cues and governing cell fate decisions. Global phospho-protein profiling, single-cell RNAseq, and functional assays revealed that MYCT1 KD causes widespread dysregulation of signaling pathways and cellular functions, including defective proliferation, whereas MYCT1 overexpression had the opposite effect. These data suggest that MYCT1 governs human HSC stemness and fate decisions by fine-tuning multiple signaling pathways through the control of endocytosis. As MYCT1 expression is downregulated in cultured HSPC, this work suggests that the inability to properly sense microenvironmental signals in cultured HSCs is a key mechanism contributing to culture-asso-

ciated HSC dysfunction that will need to be overcome to restore compromised transplantability of ex vivo expanded human HSCs.

Keywords: Hematopoietic stem cells, endocytosis, signaling

TOPIC: IMMUNE SYSTEM

621

IFN-Y PRIMING OF MESENCHYMAL STROMAL CELL SHEETS IMPROVES THEIR IMMUNOSUPPRESSIVE PARACRINE FUNCTION

Dunn, Celia M. - *Department of Biomedical Engineering, University of Utah, Salt Lake City, UT, USA*
Kameishi, Sumako - *Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT, USA*
Grainger, David - *Department of Biomedical Engineering, University of Utah, Salt Lake City, UT, USA*
Okano, Teruo - *Institute for Advanced Biomedical Sciences, Tokyo Women's Medical University, Tokyo, Japan*

Abstract: Mesenchymal stromal cells, also called mesenchymal stem cells (MSCs), are a promising treatment for immune-related diseases due to their ability to dynamically respond to and attenuate inflammation through secretion of anti-inflammatory paracrine factors. However, MSC advancement into the clinic is hindered by poor cell localization and retention when administered as suspended cell injections, hence limiting the sustained secretion of MSC therapeutic paracrine factors. Here, we assert cell sheet technology as a directly transplantable, scaffold-free cell constructed 3D tissue with superior MSC retention and localization, facilitating continuous local delivery of immune modulatory factors to reduce inflammation. The aim of this study is to enhance MSC immunomodulatory effects by combining cell sheet technology with ex vivo cytokine priming with IFN-γ, a key activator of MSC-mediated immunosuppression. Human MSCs were seeded onto temperature-responsive cultureware and cultured with 25 ng/mL IFN-γ for 2, 4, or 6 days prior to temperature-mediated detachment of readily transplantable hMSC sheets at day 6. After 6 and 4 days of IFN-γ priming, hMSC sheets significantly upregulated gene expression of IDO-1, IL-10, PTGES2, PD-L1, and HLA-DR, while only IDO-1, PD-L1, and HLA-DR were upregulated after 2 days of priming ($p < 0.05$) (n=6). Gene upregulation was directly correlated to IFN-γ priming duration, with 6 days resulting in the highest increase in gene expression. To assay immunosuppressive function, detached IFN-γ-primed hMSC sheets were indirectly cultured with stimulated peripheral blood mononuclear cells (PBMCs), and T-cell proliferation was measured at day 4. Interestingly, we observed similarly effective hMSC-mediated inhibition of T-cell proliferation within the PBMCs between all priming durations, and low inhibition of T-cell proliferation in the non-primed hMSC sheet group. The functional similarity between 2-, 4-, and 6-day primed hMSC sheets suggests that IDO-1 and PD-L1 play more critical roles in hMSC-mediated suppression of PBMC T-cell proliferation than IL-10 or PGE-2. These results introduce IFN-γ priming as a new strategy to enhance hMSC cell sheet immunomodulatory functions to advance regenerative medicine therapies for localized immune-related diseases.

Funding Source: SCM Lifesciences
Keywords: Mesenchymal Stromal Stem Cell Sheets, Immunosuppresion, Interferon-gamma priming

TOPIC: LIVER

623

HUMAN IPSC-DERIVED ORGANOID MODEL TO DELINEATE INTERPLAY OF BIOPHYSICAL AND BIOCHEMICAL PATHWAYS IN LIVER DEVELOPMENT

Jain, Ishita - *Bioengineering, University of Illinois at Urbana Champaign, Urbana, IL, USA*
Underhill, Gregory - *Bioengineering, University of Illinois at Urbana Champaign, Urbana, IL, USA*

Abstract: Controlled in vitro cell culture systems with defined multicellular geometry and microenvironmental signals have been used to reveal insights into the spatiotemporal regulation of stem cell differentiation. For instance, dynamic patterns of biomechanical cues in such in vitro systems have been associated with the spatial regulation of differentiation trajectories. Our focus is on the patterned differentiation of human iPSC-derived hepatoblasts towards a hepatocytic or cholangiocytic fate, which is a spatially-controlled differentiation process critical for liver development and regeneration. Here, we use high throughput cellular microarrays to evaluate the role of combinatorial microenvironment on patterned hepatoblast differentiation. This involves culturing human iPSC derived hepatoblasts (hiHepatoblasts) on circular shaped micropatterns with a well defined ECM, mechanical force gradient and ligand composition. We can tune the microenvironment of these microarrays in a high-throughput manner, where we can represent multiple ECM combinations in addition to ligands such as Notch ligands or E-Cadherin to the cells through well defined polyacrylamide hydrogels. Further, we modulate the biomechanics of our microarrays using different substrate stiffness polyacrylamide hydrogels, size of the micropattern and surface topography of the hydrogel. We observed iHepatoblasts arranging themselves uniquely on the circular micropatterns as a function of their specific microenvironment. They exhibited morphogenesis into unique bile duct-like 3D structures on cellular microarrays. Current work involves characterising these patterning events using immunostaining and fluorescence in-situ hybridization (FISH) of various differentiation markers. Additionally, we are delineating the underlying mechanism of this patterning and morphogenesis. This involves quantification of Notch, YAP and Cadherin signalling, all of which have been implicated in patterned liver differentiation in our previous studies. This study will help understand key events in liver development, specifically, the morphogenesis of specific 3D structures as function of the microenvironment. Further, it will give key insights into how morphogenesis can be controlled in-vitro using tailored microenvironments.

Keywords: Patterned Liver Differentiation, Microenvironment, iPSC

TOPIC: MUSCULOSKELETAL

625

MATRIX METALLOPROTEINAE ADAMTS1 REGULATES MUSCLE STEM CELL QUIESCENCE AND THE NICHE

Kim, Soochi - *Neurology, Stanford University, Stanford, CA, USA*
Kang, Jengmin - *Neurology, Stanford University, Stanford, CA, USA*

Goshayeshi, Armon - *Neurology, Stanford University, Stanford, CA, USA*
Both, Pieter - *Neurology, Stanford University, Stanford, CA, USA*
Liu, Ling - *Neurology, University of California, Los Angeles, CA, USA*
Feldman, Brian - *Pediatrics, University of California ,San Francisco, CA, USA*
Rando, Thomas - *Neurology, Stanford University, Stanford, CA, USA*

Abstract: Muscle stem cell reside within a niche that is made up of myofibers and extracellular matrix (ECM), which is critical for stem cell maintenance. Although previous reports have uncovered how signaling molecules or extracellular matrix proteins released by stem cells maintain quiescence in a cell autonomous manner, the regulation and source of molecules that maintain the stem cell niche remain largely unknown. Here we show that muscle stem cells produce matrix metalloproteinase, Adamts1 to maintain quiescence in a cell autonomous manner. Using conditional knockout model, we show that the genetic ablation of Adamts1 in muscle stem cells breaks its quiescence and impairs muscle regeneration. Global transcriptional profiling of muscle stem cells after the ablation of Adamts1 revealed several pathway changes, including enrichment of Hallmark E2F target genes set and downregulation of Notch signaling related genes. Moreover, gene ontology class analysis of differential gene expression testing, highlighted the downregulation of genes related with extracellular region and extracellular space. Collectively, our findings establish that muscle stem cells actively maintain quiescence and remodeling of the ECM is an integral process of stem cell maintenance.

Keywords: Quiescence, Adamts1, Regeneration

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BONE REGENERATION CAPACITY OF EXTRACELLULAR VESICLES ISOLATED FROM BONE MARROW-DERIVED AND ADIPOSE-DERIVED MESENCHYMAL STROMAL/STEM CELLS

Liu, Yuan - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*
Sun, Li - *Biomedical Sciences, Florida State University, Tallahassee, FL, USA*
Meckes, David - *Biomedical Sciences, Florida State University, Tallahassee, FL, USA*
Holmes, Christina - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*

Abstract: Displaying trophic and immunomodulatory effects upon transplantation, mesenchymal stromal/stem cells (MSCs) currently represent a critical part of bone regeneration research. The therapeutic effects of MSCs have recently been largely attributed to the paracrine effects of the MSC secretome, including soluble factors and extracellular vesicles (EVs). Due to the remarkable advantages of EV-based therapies, replacing or combining MSCs with their secreted EVs has begun to draw attention as a bone regeneration treatment strategy. However, it remains to be determined which MSC source produces EVs with the greatest therapeutic potential. To find the optimal MSC source for clinically translatable EV-derived bone regeneration therapies, this study aims to compare the angiogenic, osteogenic, and immunomodulatory potentials and cargo compositions of EVs isolated from the two most common clinical sources of adult MSCs, bone marrow and adipose tissue, across different passage numbers. Primary adipose- and bone marrow -derived MSCs (ASCs and BMSCs) were isolated from adult Lewis rats. Although both MSC sources secreted EVs with similar mean sizes, total protein content and average amount of protein per EV at passage 2 (P2), BMSCs showed a significantly higher EV yield per cell than ASCs ($p = 0.0368$, $n = 7$ for BMSC-EV, $n= 8$ for ASC-EV). Meanwhile, passage 4 (P4) ASC-EVs showed a significantly higher mean size than BMSC-EVs ($p=0.0185$, $n = 6$), while comparable protein per EV, total protein content, and EV yield per cell were observed. Preliminary tube formation assays employing human umbilical vein endothelial cells suggest that P2 ASC-derived EVs may have a higher angiogenic capacity than P2 BMSC-derived EVs, as indicated by increased numbers of nodes and total tube length. However, with few runs to date ($n =2$ biological runs), these increases were not statistically significant. Meanwhile P2 BMSC-EVs showed stronger immunomodulatory effects in RAW264.7 cells than P2 ASC-EVs, as suggested by increased expression of Arg1 ($p = 0.002$) and IL-10 ($p = 0.0386$) in preliminary RT-PCR data ($n= 2$ biological runs). Further in vitro comparison studies of EV angiogenic, osteogenic, and immunomodulatory capacity are currently underway to identify the optimal MSC-derived EV population for clinical bone regeneration therapies.

Keywords: Extracellular Vesicles, Mesenchymal Stromal/Stem Cells, Adipose Stem Cells

TOPIC: NEURAL

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LRP1 KNOCKOUT IN ADULT NEURAL STEM CELLS CAUSES HIPPOCAMPAL DYSFUNCTION WITH AGE, LOSS OF CXCR4 AND IMPROVEMENT IN RECOVERY AFTER STROKE

Dietert, Kristi - *Neurosurgery, UT Health San Antonio, TX, USA*
Verschelde, John - *Cell Systems and Anatomy, UT Health San Antonio, TX, USA*
Kuhn, Natalia - *Cell Systems and Anatomy, UT Health San Antonio, TX, USA*
Mahesula, Swetha - *Cell Systems and Anatomy, UT Health San Antonio, TX, USA*
Reed, Pamela - *Neurosurgery, UT Health San Antonio, TX, USA*
Sprague, Shane - *Neurosurgery, UT Health San Antonio, TX, USA*
Kokovay, Erzsebet - *Cell Systems and Anatomy, UT Health San Antonio, TX, USA*
Sayre, Naomi - *Neurosurgery, UT Health San Antonio, TX, USA*

Abstract: 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. In addition to aging, brain injury also increases risk for neurodegenerative decline, but the mechanisms driving this increased risk are unknown. Adult neurogenesis plays a vital role in mediating both recovery after injury and neurodegenerative disease. Preservation of adult neurogenesis protects from memory loss in Alzheimer's disease (AD) and enhances recovery after brain injury. This suggests that improved understanding of molecular processes underlying neurogenesis could lead to more efficacious therapies. To better understand these processes, we have begun investigating the role of low-density lipoprotein receptor related protein 1 (LRP1) in adult neurogenesis. Despite being amply expressed in adult NSCs, the role LRP1 plays in neurogenesis is understudied. LRP1 is well-known for its shrouded, yet established role as a player in neurodegeneration. LRP1 is involved in trafficking ApoE4, amyloid beta,

and tau – all of which each play a role in AD pathogenesis. Using a Nestin-Cre inducible mouse model to knockout LRP1 in NSCs of adult mice, we are investigating the effects of LRP1 loss both in recovery after stroke and during naïve brain aging. We found that LRP1 knockout caused a 10-fold loss of CXCR4 expression and deficits in ischemia-stimulated migration from the subventricular zone. However, these mice displayed improvements in functional recovery and lesion size 2 weeks post-stroke. In contrast with this apparent benefit in short term recovery after injury, we have found that mice lacking NSC LRP1 display a variety of behavioral deficits at 9 months of age (6 months after knockout) that suggest dysregulated hippocampal function. Specifically, mice lacking NSC LRP1 exhibit hippocampal-dependent memory deficits with a suggestion of a loss of pattern separation – a behavior explicitly dependent on hippocampal neurogenesis. Ongoing research is being conducted to elucidate the mechanistic underpinnings of these changes and their relation to cognitive decline and recovery after injury.

Funding Source: Training Grant TL1 TR002647 – TST TL1 Owen's Foundation Veteran's Administration CDA2
Keywords: Adult neurogenesis, Stroke, Memory loss

TOPIC: PANCREAS

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TRACING THE FATE OF CYTOKERATIN 19+ CELLS DURING BETA-CELL REGENERATION STIMULATED BY MULTIPOTENT STROMAL CELL SECRETED EFFECTORS.

Rasiwala, Nazihah - *Physiology and Pharmacology, University of Western Ontario, London, ON, Canada*
Bell, Gillian - *Physiology and Pharmacology, University of Western Ontario, London, ON, Canada*
Hess, David - *Physiology and Pharmacology, Robarts Research Institute- University of Western Ontario, London, ON, Canada*

Abstract: During pancreas development or after pancreatic ductal ligation in adult mice, new beta cells are generated from ductal-resident precursor cells. Thus, the induction of islet neogenesis in situ represents a promising approach to replenish beta cells lost during diabetes. Our lab has previously shown that human multipotent stromal cells (hMSC) secrete pro-regenerative factors that foster a niche for islet regeneration. Intra-pancreatic (iPAN) injection of hMSC conditioned media generated during Wnt-pathway stimulation (Wnt+ CdM) reduced hyperglycemia in streptozotocin (STZ) treated NOD/SCID mice via neo-islet formation adjacent to hyperproliferative cytokeratin 19 (CK19)+ ducts. Herein, we used the CK19-CreERT Rosa26-mTomato lineage tracing mouse model to delineate islet regenerative mechanisms stimulated by Wnt+ CdM. We hypothesized that iPAN injection of Wnt+ CdM would induce CK19+ ductal-derived precursor cell conversion to insulin+ beta cells in the adult mouse pancreas. Tamoxifen treatment (480 mg/kg) in 8-10 week old mice induced tdTomato expression in pancreatic ductal and acinar cells in a mosaic fashion. After STZ treatment (50 mg/kg x 5 days), iPAN injection of 4 µg secreted protein from Wnt+ CdM increased beta-cell mass and islet number ($p< 0.05$), but did not reduce non-fasted blood glucose levels at 11 days post-injection compared to basal media controls. Flow cytometry on dissociated pancreas tissue revealed tdTomato+/insulin+ cells were increased ($p< 0.05$) in mice injected with Wnt+ CdM (0.58±0.34%) compared to basal media (0.16±0.10%), suggesting neogenic beta cells originated from labelled CK19+ ductal or acinar cells. To augment the maturation of neogenic beta cells, we supplemented Wnt+ hMSC with heat-inactivated serum (HIS) in culture, which increased regenerative protein production ~25-fold. Initial studies of iPAN injection of 200-300 µg secreted protein from Wnt+ CdM + HIS improved non-fasted blood glucose, beta-cell mass, and islet number compared to injection of basal media + HIS. We have shown for the first time that beta-cell neogenesis from a CK19+ cell origin was stimulated by Wnt+ CdM in an immune-competent mouse model of islet regeneration, thus contributing to the development of a cell-free regenerative therapy for diabetes.

Funding Source: This research project is supported by the Canadian Institutes of Health Research and the Juvenile Diabetes Research Foundation.
Keywords: Islet regeneration, Diabetes, Multipotent stromal cells

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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LL-37 INCREASES IL-10 PRODUCTION BY HUMAN MESENCHYMAL STEM CELLS AND ENHANCES ITS THERAPUETIC EFFECTS AGAINST POLYCYSTIC OVARY SYNDROME

Park, Hang-soo - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Seok, Jin - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Liakath Ali, Farzana Begum - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Mousaei Ghasroldasht, Mohammad - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Al-Hendy, Ayman - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*

Abstract: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age associated with low-grade chronic inflammation and hyperandrogenemia. Previous studies have demonstrated the therapeutic efficacy of human mesenchymal stem cells (hMSCs) for PCOS. In our previous study, we suggested that hMSC reverse PCOS condition through its secreting factors such as IL-10 by reducing inflammation, inhibit androgen production, and regulate metabolic pathways. Recent study reported that immunomodulatory functions of cathelicidin LL-37 (LL-37) which is one of the host defense peptides (HDPs) and the sole member of the cathelicidin family found in humans. It has been reported that LL-37 promotes higher levels of anti-inflammatory factors including IL-10, and boosts the immune suppressive function of MSCs. Based on this finding and our previous result in PCOS, we hypothesized that boosting IL-10 production in hMSC using LL-37 could enhance the therapeutic effect on PCOS treatment. Here, we improved the therapeutic effect of hMSC on our in vitro PCOS models H295R cells through enhancing IL-10 production in MSC via non-genetic stimulation using LL-37. For in vitro experiment, we treated conditioned media from LL37 primed hMSC to androgen producing H293R cells, and analyzed androgen producing gene expression. The effect of hMSC on androgen producing cells was assessed by monitoring cell proliferation (cell counting), steroidogenic gene expression (quantitative real-time polymerase chain reaction [qRT-PCR]). We found that LL-37 treatment was successfully increased the IL-10 production in MSC around 2-fold (189.6 ± 14.2 pg/ml). In addition, conditioned

media from these primed MSC shows higher regulatory effect on H295R cells such as proliferation, viability, and androgen producing gene expression. Our study demonstrates the efficacy of MSC for the treatment of PCOS conditions could be enhanced through stimulating IL-10 production using LL-37. Our work suggests an efficient and novel therapeutic option using stimulated MSC without genetic modification for women with PCOS. Further preclinical study with animal model and pilot clinical trials are required to further evaluate and validate this novel treatment option for this common female metabolic/reproductive disorder.

Funding Source: This study supported by start-up fund of the University of Chicago (AA).
Keywords: Mesenchymal stem cell, IL-10, female infertility

TOPIC: PLURIPOTENT STEM CELLS

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EXTRACELLULAR VESICLES MEDIATED CELL-CELL COMMUNICATION IS REQUIRED FOR PLANARIAN TISSUE MAINTENANCE

Sasidharan, Vidyanand - *Stowers Institute, Kansas City, MO, USA*
Deng, Fengyan - *Histology, Stowers, Kansas City, MO, USA*
Yi, Kexi - *Microscopy, Stowers Institute, Kansas City, MO, USA*
Swanson, Selene - *Proteomics, Stowers Institute, Kansas City, MO, USA*
Yu, Zulin - *Microscopy, Stowers Institute, Kansas City, MO, USA*
Mckinney, Sean - *Microscopy, Stowers Institute, Kansas City, MO, USA*
Alvarado, Alejandro S. - *Stowers, Kansas City, MO, USA*

Abstract: Upon amputation, planarian stem cells (neoblasts) first exhibit body-wide proliferation followed by mobilization and localized proliferation of a subset of cells to the vicinity of the wound. These coordinated steps are critical to blastema formation and suggest an important role for intercellular communication during wound healing and tissue regeneration. Yet, little is known about the factors affecting the observed cellular biology. Recently, Extracellular Vesicles (EVs) have been shown to play a role in cell-cell communication in multiple systems. EVs are lipid bi-layered structures known to carry a wide variety of biomolecules between cells and have been shown to play roles in cancer, metabolic disorders, and neurological disorders. Here we show the importance of EV biology in planarian regeneration. RNAi on exosome biogenesis pathway candidates leads to lethality in regenerating and intact animals. We performed proteomics and RNA sequencing to characterize the cargo packaged in EVs. We could perturb the migration of stem cells in regenerating animals by performing RNAi on genes packaged inside the EVs. Cultured planarian cells showed two ways of EV transport among cells: 1) by secreting EVs; and 2) through Tunneling Nanotubules (TNTs). Electron micrograph images showed the accumulation and transport of EVs between cells using TNTs. Thus, studying EV biology could help us further understand modes of cell-cell communication in regenerating model systems.

Funding Source: HHMI, Stowers Institute for Medical Research
Keywords: regeneration, extracellular vesicles, nanofilaments

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

761

THE METABOLISM CHANGES DURING LONG-TERM CULTURE OF ADIPOSE DERIVED STEM/STROMAL CELLS UNDER 5% OXYGEN CONCENTRATION

Rybkowska, Paulina - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warszawa, Poland*
Dymkowska, Dorota - *Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland*
Zabłocki, Krzysztof - *Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland*
Sarnowska, Anna - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warsaw, Poland*

Abstract: The plasticity and regenerative capacity of human mesenchymal stem/stromal cells (MSC) has been widely studied, however, the mechanisms underlying their most beneficial effects are still not completely clear. According to our previous experience, the critical factor that influences stem cell properties and differentiational potential is the oxygen concentration. In this project, we determined how the basal energy metabolism of adipose tissue-derived stem cells (ASC) changes during long-term in vitro culture under different oxygen concentration and whether it corresponds with cell properties. We chose three time points: early - 2, middle - 6, and late 12 cell passage, and we compered two oxygen concentrations: 5% O2 (physioxic) and atmospheric - 21% O2. We discovered, that under physioxic conditions ASC have significantly higher proliferation potential (estimated the population doubling time, WST assay and Ki67 expression) and migration capacity (scratch test) compared to the culture provided in 21%. As culture time progressed, both parameters decreased. Physioxic-cultured condition increased also the expression of Stemness Related Transcriptional Factors (SRTF) such as Nanog and Oct ¾ . ASC progressively lose expression of above mentioned genes at passage 6 and 12 in both oxygen culture conditions. Metabolic analysis provided with Seahorse XF revealed that ASC cultured in physioxic conditions (5%O2) compared with cells cultured in 21% O2 have lower oxygen consumption rate (OCR) that corresponds with mitochondrial respiration capability and higher extracellular acidification rate (ECAR) that is directly related with anaerobic glycolysis. ASC cultured under 5% O2 exhibit higher expression of enzyme's genes such as LDHA and PDHA mainly related to glycolysis and its auxiliary pathways such as the pentose-phosphate pathway. Our results may suggest that ASC at low oxygen concentrations reduce mitochondrial respiration by converting their central metabolism to anaerobic glycolysis. Moreover, obtained results indicate that this metabolic reconfiguration correlates with crucial cells properties.

Funding Source: 2018/31/N/NZ4/03275
Keywords: mezenchymal stem cells, metabolism, physioxia

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

763

YOLK SAC-DERIVED PROGENITORS ARE A LOCAL SOURCE OF ENDOTHELIAL CELLS AND MACROPHAGES IN ADULT MUSCLE AND PROMOTE PERFUSION RECOVERY AND MYOCYTE REPAIR AFTER ISCHAEMIA

Tran, Dang Xuan Anh - *Vascular Research Centre, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia*
Williamson, Anna - *Vascular Research Centre, SAHMRI, Adelaide, Australia*
Liyanage, Sanuri - *Vascular Research Centre, SAHMRI, Adelaide, Australia*
Hassanshahi, Mohammadhossein - *Vascular Research Centre, SAHMRI, Adelaide, Australia*
Bursill, Christina - *Vascular Research Centre, SAHMRI, Adelaide, Australia*
Psaltis, Peter - *Vascular Research Centre, SAHMRI, Adelaide, Australia*

Abstract: Macrophages and endothelial cells (ECs) play key, interlinked roles in regulating inflammation and neovascularisation involved in tissue recovery after ischaemic insults. Recent studies indicate that both cell types may share a common developmental origin from erythromyeloid progenitor cells (EMPs) in the extra-embryonic yolk sac (YS). We recently discovered YS EMP-derived Lin-CD45+/LoSca-1+c-Kit+CX3CR1+CSF1R+ endothelial-macrophage (EndoMac) progenitors in murine aorta. Here, we investigated the role of these cells in steady-state and ischaemic skeletal muscle. Csf1rMer-iCre-Mer x RosamT/mG mice were administered tamoxifen at E8.5 to induce GFP expression in YS EMP-derived cells. Then gastrocnemius and quadriceps were harvested from adult mice and studied by confocal microscopy, which revealed the presence of GFP+ c-Kit+ progenitors, VE-Cadherin+ and CD31+ ECs, and CD68+ macrophages in the tissues. Following surgery to induce hindlimb ischaemia, YS-derived EndoMac progenitors underwent marked proliferative expansion in ischaemic muscle within the first 24 hrs, whereas macrophages and ECs expanded later. EndoMac progenitors from ischaemic muscle had greater capacity to form macrophage colony-forming units in methylcellulose and angiogenic cords in MatrigelTM, compared to progenitors from non-ischaemic muscle. Similar results were obtained when progenitors were cultured under hypoxia compared to normoxia. Finally, EndoMac progenitors (~1.5x104) from muscle of donor GFP mice were injected into hindlimbs of C57BL/6 recipients immediately after ischaemic surgery. By comparison to vehicle control, progenitors significantly increased perfusion recovery and capillary density. Moreover, they resulted in greater myocyte size and density. The majority of engrafted GFP+ progenitors were found to form ECs (~50%) and macrophages (~38%), along with host-perfused neovessels. This study identifies the presence of YS-derived EndoMac progenitors in adult muscle. These bipotent, vasculogenic cells proliferate and expand early after ischaemia and form new ECs and macrophages to promote perfusion recovery and myocyte repair.

Funding Source: National Health and Medical Research Council (NHMRC)
Keywords: Yolk sac-derived progenitor cells, Endothelial cells and macrophages, Ischemia

TOPIC: EPITHELIAL SKIN

765

INTEREST OF IL-β-PRIMED MESENCHYMAL STROMALS CELLS AND THEIR SECRETED PRODUCTS FOR BURN WOUND THERAPIES

Trouillas, Marina - *Cell and Tissue Therapy Unit, French Armed-forces Biomedical Research Institute (IRBA) / UMR-MD-U1197, Clamart, France*
Dedier, Marianne - *Cell and Tissue Therapy Unit, French Armed-forces Biomedical Research Institute (IRBA) / UMR-MD-U1197, Clamart, France*
Nivet, Muriel - *Cell and Tissue Therapy Unit, French Armed-forces Biomedical Research Institute (IRBA) / UMR-MD-U1197, Clamart, France*
Baus, Arnaud - *Plastic Surgery, Percy Hospital, Clamart, France*
Banzet, Sebastien - *Cell and Tissue Therapy Unit, French Armed-forces Biomedical Research Institute (IRBA) / UMR-MD-U1197, Clamart, France*

Abstract: Rapid wound coverage and definitive closure of burn lesions is one of the determining factors for the survival of major burn patients. Despite several alternatives using skin tissue engineering and regenerative medicine, current therapeutic solutions remained unsatisfactory and could lead to severe infections, skin dysfunction and pathological scar. Mesenchymal Stromal Cells (MSCs) have aroused growing therapeutic interest for skin repair, thanks to their trophic, their immunomodulatory, their cell matrix remodeling, and their pro-angiogenic properties. MSC seem to exert their activity mainly by the secretion of free bioactive molecules and extracellular vesicles. Discovery of their high plasticity has opened up new therapeutic perspectives with the use of molecular, cellular and physical stimuli also called “priming” to enhance their activity. Recent work from our laboratory shows that the use of IL-1β priming on MSC improves their wound healing potential in vitro and in vivo in a skin excision model. In this study, we investigated whether IL-1β-primed MSC or their secretion could improve autologous skin graft take in a severe burn rat model. Syngeneic IL-1β-primed MSC, derived from bone marrow, or their secreted products concentrated by tangential flow filtration were evaluated in a 3rd degree rat burn model. This model reproduced surgical management of severe burn patient with in a rapid excision of burned tissue and autologous split-thickness skin graft application to ratio 1:6 concomitants with the subcutaneous injection of MSC or their secreted products. Our results showed that IL-1β-primed-MSC administration enhanced total wound healing reepithelialization and favored vascularization. We hope that this study will help find an innovative MSC based-therapy for severe burn patients.

Keywords: Severe burns, Mesenchymal stromal cells, Priming

TOPIC: HEMATOPOIETIC SYSTEM

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SINGLE CELL ANALYSIS PROVIDES INSIGHT INTO HAEMATOPOIETIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Nnadi, Nneka C. - *Stem Cell, Institute for Stem Cell Research - University of Edinburgh, UK*
MacLean, Adam - *USC Dornsife College of Letters, Arts and Sciences, University of Southern California, Los Angeles, CA, USA*
Medvinsky, Alexander - *Institute of Stem Cell Research, University of Edinburgh, UK*

Abstract: One of the challenges in regenerative medicine remains generating functional Haematopoietic stem cells (HSCs) of clinical quantity. Utilizing data from single cell RNA sequencing (scRNA-seq) of human embryonic stem cells (hESC) derived cells, we explored the differences that exist between in vitro and in vivo haematopoiesis, with the aim that understanding these differences could help improve future chances of generating HSCs in vitro. By investigating dynamic gene expression of cell populations emerging during hESC differentiation, we observed that the haematogenic endothelium capable of generating the haematopoietic progenitors emerge as early as day 6 of differentiation. Also, we identified that priming of the arterial endothelial cells to the haematogenic endothelium, and subsequent emergence of haematopoietic progenitors is associated with an increase in ribosomal and mitochondrial activity. Lastly, a comparative analysis of the hESCs generated dataset to publicly available dataset from embryonic haematopoietic tissues revealed that in vitro directed haematopoiesis, associated with mitochondrial gene activity, is likely mimicking events of the yolk sac haematopoiesis, rather than the wave of haematopoiesis resulting in the emergence of HSCs that take place in the AGM region. Altogether, our data suggests that the increased mitochondrial activity, may be contributing to the challenge in generating HSCs from hESCs in a dish.

Funding Source: Presidential Special Scholarship for Innovation and Development (PRESSID)
Keywords: single cell RNA sequencing, human ESC derived haematopoiesis, Mitochondrial activity

TOPIC: KIDNEY

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REGENERATION OF MULTIFUNCTIONAL KIDNEYS BY THE I-DROP SYSTEM USING THE MECHANISM OF KIDNEY DEVELOPMENT IN ANIMAL FETUSES

Saito, Yatsumu - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Yamanaka, Shuichiro - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Matsui, Kenji - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Matsumoto, Naoto - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Takamura, Tsuyoshi - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Fujimoto, Toshinari - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Tajiri, Susumu - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Matsumoto, Kei - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*
Kobayashi, Eiji - *Department of Kidney Regeneration, The Jikei University School of Medicine, Tokyo, Japan*
Yokoo, Takashi - *Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan*

Abstract: An increase in the cases of renal failure worldwide has led to a shortage of organs for transplantation. Thus, the generation of new kidneys through regenerative medicine is a viable solution. However, it is difficult to generate renal stroma, which play a crucial role in the maintenance of kidney structure, endocrine function, and kidney development, in vitro from pluripotent stem cells; the regeneration of a mature whole kidney has not been achieved to date. Our goal was to generate human kidneys using the nephrogenic environment (niche) of heterologous fetal animals, a method that could provide an appropriate in vivo environment for renal stromal differentiation. We attempted to generate renal stroma by applying mouse renal developmental signals to rat stromal progenitor cells (SPCs) and to simultaneously generate nephrons and renal stroma using an interspecies dual replacement of the progenitor (i-DROP) system. In this system, two progenitors of heterologous fetuses, nephron progenitor cells (NPCs) and SPCs, were genetically modified to allow their removal by drugs and their replacement by transplanted progenitors. The SPCs of SD-TG (CAG-EGFP) rats (GFP rats) were injected into the metanephros of Foxd1-iDTR (host SPC-removal model) mice, whereas the NPCs and SPCs of GFP rats were injected into the metanephros of Six2/Foxd1-iDTR (host NPC& SPC-removal model) mice. The metanephros were organ cultured or transplanted into the retroperitoneum of NOD/Shi-scid/IL-2Rγnull mice. The specimens were evaluated via immunofluorescence staining. In the SPC-removal model, mice SPCs were replaced with rat SPCs in vitro; rat stroma was extensively regenerated with a high chimerism rate. Moreover, stromal cells with endocrine functions (renin and erythropoietin production), were generated in vivo. In the two progenitor-removal models, the cap mesenchyme was reconstituted in vitro using mouse ureteric buds and rat NPCs and SPCs. Furthermore, simultaneous in vivo generation of rat nephrons and stroma, with glomerular filtration and tubular reabsorption capacity, was successfully achieved. Therefore, the newly developed i-DROP system shows potential for use in the regeneration of functional human kidneys, in terms of urine production and endocrine functions, in the environment of animal kidney development.

Funding Source: Japan Agency for Medical Research and Development, Japan Society for the Promotion of Science, Yukiko Ishibashi Foundation
Keywords: Kidney regeneration, Chimera, Xenotransplantation

TOPIC: NEURAL

771

IMPLANTATION OF UNDIFFERENTIATED HUMAN NEURONAL PROGENITOR CELLS (NPCS) IN THE ACUTE PHASE OF 6-OHDA-INDUCED NIGRAL DEGENERATION ALLOWS ADDRESSING OF THE PROGRESSIVE NATURE OF PARKINSONS DISEASE

Kopyov, Alex - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Marhamati, Neda - *Research and Development, Celavie Biosciences LLC, Santa Barbara, CA, USA*
Shatzen, Edward - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Ochoa, Jessica - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Mamilapally, Swapna - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Freise, Alyssa - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Lazarz, Victoria - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
Bondelid, Karina - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*
McNulty, Nikkole - *Research and Development, Celavie Biosciences LLC, Sylmar, CA, USA*
Bock, Jason - *Statistical Consultant, Newport Beach, CA, USA*
Wells, Christine - *Statistical Consultant, Newbury Park, CA, USA*
Kopyov, Oleg - *Research and Development, Celavie Biosciences LLC, Oxnard, CA, USA*

Abstract: The 6-OHDA model of dopamine (DA) deficiency has been successfully used to study regenerative effects of pre-differentiated neurons for two decades. The traditional static, design specifies stem cells be implanted two weeks or longer after 6-OHDA injection. By this time the 6-OHDA has destroyed all of the DA neurons at the target location making this design not suitable for undifferentiated cells that rely on microenvironmental cues to formulate a response. There is no ongoing neuronal death, and thus no microenvironmental cues from the progressive neurodegeneration two weeks post 6-OHDA injection. Our previous studies demonstrate a very modest response to undifferentiated cells implanted 2 weeks after 6-OHDA injection (unpublished data). To capture the NPC response to the pathological microenvironmental cues produced by 6-OHDA nigral degeneration, we implanted the undifferentiated cells within 24 hours of the 6-OHDA injection. This is the period when the DA neuron death is still ongoing and stem cells are given an opportunity to interact with the microenvironmental cues. We injected 6-OHDA into rats' right Substantia Nigra (SN). Undifferentiated NPCs were implanted into the right putamen on the day of the 6-OHDA injection. A second dose of NPCs was implanted 1mm above the right SN one day after. The control group received vehicle instead of cells at both sites. Both groups were tested for rotational behavior by apomorphine injection at monthly intervals. At the conclusion of the experiment, brains were frozen-sectioned and stained for DA neurons with anti-Tyrosine Hydroxylase antibodies. The rats that received stem cell injections demonstrated significantly fewer (ANOVA, $F[52,94] = 10.82$, $p < 0.001$) apomorphine-induced rotations than vehicle through the 4 months of the follow-up. Frozen sections of the SN regions of the brains of the control animals exhibited complete destruction of the right SN. Anatomically matched sections from the experimental group revealed a near-complete repopulation of right SN with TH-positive neurons in experimental animals. The study suggests that implanting the cells in the acute phase of the 6-OHDA-induced neurodegeneration is a viable experimental design for studying the regenerative effects of the undifferentiated stem cells.

Keywords: Undifferentiated stem cells, Parkinson's Disease, 6-hydroxydopamine

TOPIC: PANCREAS

773

MECHANISMS OF ER-STRESS-INDUCED HUMAN BETA-CELL DEATH

Martinez, Tomas R. - *Biotechnology and Bioinformatics, California State University, Channel Islands, Oxnard, CA, USA*
Sander, Maike - *Pediatrics, University of California, San Diego, CA, USA*
Zhu, Han - *Pediatrics, University of California, San Diego, CA, USA*
Okino, Mei-Lin - *Pediatrics, University of California, San Diego, CA, USA*

Abstract: β-cell dysfunction and β-cell loss are hallmark features of both type 1 (T1D) and type 2 diabetes (T2D). In T1D the immune system plays a critical role in disease pathogenesis; however, it is increasingly clear that the β-cells themselves also contribute. A current working model is that aberrant immune cell activity as well as increased β-cell sensitivity to inflammatory and metabolic stressors both contribute to T1D. In T2D, β-cell failure is thought to be caused by a combination of genetic factors, insulin resistance, and systemic inflammation. In both diseases, environmental stimuli such as proinflammatory cytokines and hyperglycemia, activate the β-cell endoplasmic reticulum (ER) stress response, also called the unfolded protein response (UPR). Acute activation of the UPR leads to an adaptive response through transient halting of protein translation and increased transcription of ER chaperones and lipid synthesis genes to increase ER capacity. Chronic or prolonged UPR activation results in induction of pro-apoptotic genes, leading to cell death. Therefore, it is possible that intrinsic differences in how β-cells respond to triggers of the ER stress response contribute to diabetes susceptibility and explain genetic risk for both T1D and T2D at the level of β-cells. However, the mechanisms and molecular effectors regulating β-cell viability upon activation of the ER stress response are poorly understood. To globally identify genes that regulate β-cell survival under ER stress, I will conduct a CRISPR/Cas9 loss-of-function screen by treating β-cells derived from human pluripotent stem cells (SC-β-cells) with the ER stress inducer thapsigargin. For this purpose, I have confirmed that thapsigargin triggers similar transcriptional changes in SC-β-cells as in primary β-cells in human islets. I have determined that 1 μM thapsigargin causes a 40% reduction in SC-β-cell numbers by 48 hours of treatment and will use this treatment scheme in the CRISPR/Cas9 loss-of-function screen. Genes identified in the screen will provide insights into mechanisms and relevant molecular effectors controlling β-cell death upon activation of the ER stress response.

Funding Source: This project is funded by: U01DK120429 and R01DK122607
Keywords: β-cell survival under ER stress, CRISPR/Cas9 loss-of-function screen, Type 1 (T1D) and type 2 diabetes (T2D)

TOPIC: PLURIPOTENT STEM CELLS

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APELIN RECEPTOR (APLNR) SIGNALING AS A PLAYER OF MESODERM CELL MOVEMENT AND MESENCHYMAL STEM CELL (MSC) DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

Dogan, Aysegul - *Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey*
Şişli, Hatice Burcu - *Genetics and Bioengineering, Yeditepe University, Kadıköy/İstanbul, Turkey*
Şenkal, Selinay - *Genetics and Bioengineering, Yeditepe University, Kadıköy/İstanbul, Turkey*
Hayal, Taha Bartu - *Genetics and Bioengineering, Yeditepe University, Kadıköy/İstanbul, Turkey*
Bulut, Ezgi - *Genetics and Bioengineering, Yeditepe University, Kadıköy/İstanbul, Turkey*
Şahin, Fikrettin - *Genetics and Bioengineering, Yeditepe University, Kadıköy/İstanbul, Turkey*

Abstract: Identification of regulatory pathways in differentiation and cell fate decision during embryonic development is not only important for embryogenesis but also required for pluripotent stem cell based regenerative medicine applications. Generation of safe and efficient cell sources such as Mesenchymal stem cells (MSCs) became one of the indispensable therapeutic tools in cell therapy. Generation of MSCs with high proliferation and differentiation potential from PSCs, which have unlimited proliferation capacity, may eliminate these limitations. Since mesoderm layer give rise to most of the MSCs, signaling events related to its migration and differentiation hold a great significance. MSCs were reported to arise from Aplnr+ populations of hESC-derived mesoderm cells. Also, being a target gene of Wnt signaling, which is one of the masterminds behind gastrulation movements and morphogenetic events; Aplnr signaling may be a therapeutic tool for cell therapy. Here, we generated MSCs from hESC-derived mesoderm cells and investigated role Aplnr signaling during mesoderm cell migration and differentiation. Aplnr signaling components were knocked down, knocked out, induced by a peptide, and overexpressed in during mesoderm and MSC differentiation. Aplnr signaling was induced at various time periods during MSC differentiation protocol from hESC. MSCs were characterized by cell phenotype, plastic adherence capacity, MSC marker gene and protein expression, self-renewal, and differentiation potential. After 24 days, hESCs were successfully differentiated to MSCs, with fibroblast cell morphology. Continuous administration of Apelin during MSC differentiation led to overexpression of MSC markers. Aplnr signaling activity not only increased proliferation, self-renew and differentiation potential of MSCs but also enhanced migration of mesoderm cells. RNA sequencing and protein array experiments were conducted to explore the underlying molecular mechanism. Pathways regulating mesoderm tissue specification and MSC differentiation were identified. These results reveal regulatory role of Aplnr signaling in mesoderm cell movement and MSCs differentiation from mesoderm layer.

Funding Source: This study was supported by TUBITAK 2232 International Fellowship for Outstanding Researchers Program (Project no: 118C186).
Keywords: Mesoderm, Mesenchymal Stem Cell, Apelin

POSTER SESSION I: EVEN

7:30 PM – 8:30 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

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GENERATION OF CARDIAC ORGANOIDS FROM PATIENT-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

Solvang, Vanessa - *Department of Clinical and Molecular Medicine, The Norwegian University of Science and Technology, Trondheim, Norway*

Abstract: Heart diseases are a leading cause of mortality worldwide. A robust and reproducible in vitro heart model is therefore of major clinical value in studying heart diseases and the molecular mechanisms governing the human heart. Human induced pluripotent stem cells (hiPSCs) derived from human tissue have increasingly gained attention in the development of such model systems because of their non-invasive production and broad differentiation capacity. Of special interest, is the differentiation of hiPSCs into three-dimensional heart structures, commonly referred to as cardiac organoids. Cardiac organoids have an improved ability to mimic real heart tissue compared with traditional monolayer cell cultures due to a higher degree of cell-cell interactions and cell organization. Optimal cardiac organoids will provide great tools in modeling heart diseases and cardiogenesis, drug screening, cardiotoxicology testing, and regenerative cell-based or gene therapies. Here we present a stepwise procedure for generating cardiac organoids: From the harvesting a skin biopsy from a patient, through the reprogramming of patient fibroblasts into hiPSCs, and ultimately the differentiation of hiPSCs into cardiac organoids with optimized medium cocktails. Each step in the process was thoroughly analyzed with quantitative PCR and immunocytochemistry to verify the presence of key gene and protein markers characterizing the main cell stages. The generated cardiac organoids showed rhythmical and spontaneous contractions and were predominantly composed of cardiomyocytes indicated by high expression of the cardiac markers TNNT2 and ACTC1. Additional analysis also revealed the presence of VIM+ cardiac fibroblasts and WT1+ epicardial cells. Although cardiac organoids can be generated by the method presented, there is still room for improvement of the model in terms of cell complexity, purity, and internal structuring.

Keywords: Cardiac organoids, Cardiogenic differentiation, hiPSCs

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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METABOLIC INFLUENCE ON TROPHECTODERM SPECIFICATION AND MAINTENANCE

Mohanty, Sujata - *Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India*
Banerjee, Arup - *Department of Virology, Regional Centre for Biotechnology, Faridabad, India*
Gupta, Suchi - *Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India*
Krishnakumar, Vishnu - *Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India*
Soni, Naina - *Department of Virology, Regional Centre for Biotechnology, Faridabad, India*
Rao, E Pranshu - *Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India*
Sharma, Yashvi - *Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India*

Abstract: Extracellular vesicles (EVs) are emerging as crucial intercellular messengers that contribute to the physiological processes. EVs contain numerous functional proteins and nucleic acids derived from their parent cells and have different roles depending on their origin. Functionally, EVs transfer these biological materials from the parent cell to the recipient and thus exhibit a novel therapeutic platform for delivering therapeutic molecules to the target tissue. In this regard, EVs derived from stem cells such as Mesenchymal Stem Cells and iPSCs have demonstrated a higher ability to benefit regenerative medicine. Even though these stem cells share some common properties, due to the differences in their origin (cell sources, the hierarchy of potency, etc) the EVs cargo profiling and functionality may vary. Accumulating evidence has shown many elaborated studies on miRNAs, but in-depth knowledge on EV proteomics is limited. Therefore, in this study, we have performed an iTRAQ-based proteomic analysis for comprehensive and quantitative evaluation of EVs derived from iPSCs and different tissue-specific MSCs. Bioinformatics analysis revealed 223 differentially expressed proteins in these EVs; however, more exclusive proteins with higher protein expressions were from Wharton's jelly MSC-EV. Further, amongst these proteins, 113 proteins were abundant in MSCs EVs while others were shared by MSC-EVs and iPSC-EVs. Moreover, these proteins were categorized for their functional regenerative role in bone formation, immune regulation, wound healing, cardio-protection, neuroprotection, and hepato-protection. Collectively, the results of our proteomic analysis show that MSC derived EVs contain a robust profile of proteins with higher expression as compared to that of iPSCs.

Funding Source: The work done in this study was generously supported by BIRAC and Department of Biotechnology, India.
Keywords: Extracellular vesicles, Proteomic profiling, Wharton's jelly MSC & iPSCs

TOPIC: EARLY EMBRYO

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METABOLIC INFLUENCE ON TROPHECTODERM SPECIFICATION AND MAINTENANCE

Casòliba-Melich, Arnau - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Pladevall-Morera, David - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Kafkia, Eleni - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Bages-Arnal, Sandra - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Zylicz, Jan - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Denmark*

Abstract: Early development is accompanied by drastic changes in gene expression, transcription factor networks, metabolic pathways and epigenetic modifications. While these regulatory processes are functionally coupled, it remains unclear how they are coordinated and what function they play during development. In this project, I address this question by focusing on the role of metabolism during first lineage decision of development. Indeed, once the blastomeres decide between the inner cell mass (ICM) and trophectoderm (TE) fate they also activate drastically different metabolic networks. Concomitantly, some of the tricarboxylic acid (TCA) cycle enzymes can be identified in the nucleus. However, it is unclear what is the role of these metabolic changes in establishing and maintaining the TE program? Metabolism directly regulates chromatin marks, but how important is this for regulating specific transcriptional programs? Here, by using acute loss-of-function systems I uncover that some metabolic enzymes regulate specific chromatin states in embryonic stem cells and trophoblast stem cells (ESCs and TSCs), the in vitro models of the ICM and TE respectively. Now the question remains how such specificity is achieved in terms of chromatin marks and genes affected. This work aims at uncovering the molecular mechanism of epi-metabolic regulation and its function in regulating discrete cell-fate changes.

Funding Source: Funding Source: This research is supported by: The Novo Nordisk Foundation reNEW grant number NNF21CC0073729, Danmarks Frie Forskingsfond grant number 0169-00031B and Lundbeck Fonden grant number R345-2020-1497.
Keywords: Trophectoderm specification, Metabolism, Epigenetics

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DEVELOPMENTAL COMPETENCE OF PORCINE TETRAPLOID (4N) EMBRYOS USING VARIOUS METHODS.

Lee, Joohyeong - *Institute for Stem Cell & Regenerative Medicine (ISCRM), Laboratory of Veterinary Embryology and Biotechnology (VETEMBIO) and College of Veterinary Medicine, Chungbuk National University, Cheongju-si, Korea*
Hyun, Sang-Hwan - *Institute for Stem Cell & Regenerative Medicine (ISCRM), Laboratory of Veterinary Embryology and Biotechnology (VETEMBIO), Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju-si, Korea*

Lee, Eunsong - *Kangwon National University, Chuncheon-si, Korea*

Abstract: The tetraploid (4N) embryo has been widely used for animal reproduction. It has been reported that in chimeric embryos reconstituted with diploid (2N) and 4N blastomeres, 2N cells contributed to the inner cell mass whereas most 4N cells contributed to the trophectoderm. Furthermore, embryonic stem cells that do not form trophoblasts can aggregate with 4N embryos to produce complete live offspring. Thus, 4N embryos can support the differentiation of 2N cells or 2N blastomere. This study aimed to produce porcine 4N embryos using various methods and evaluate theirs in vitro developmental competence. In this study, three methods were investigated to produce 4N embryos. First, 4N embryos were produced by electro-fusion of 2-cell stage parthenogenetic (PA) embryos (FUTP). Second, 2N somatic cell was injected into the mature oocyte and fused to produce 4N embryos (CITP). Third, oocytes were matured with Cytochalasin B (CB) for the late 22 hours of in vitro maturation to inhibit the first polar body (PB1). Thereafter, non-PB1 oocytes were treated with CB for 4 hours after PA (CBTP). As a result, it was confirmed that the extrusion of the PB1 was inhibited in 96.7% of oocytes. When the cleavage rate of embryos was observed 24 hours after activation, there was no significant difference in the control (75.6%) and all treatment groups (70.0-70.8%). At 24 hours after activation, only 2-cell stage embryos were selected and cultured for an additional 6 days to investigate the proportion of embryos developing to the blastocysts. As a result, the blastocyst development rate in the CBTP group (66.9%) was not significantly different from that of the control group, but the FUTP (54.5%) and CITP (38.3%) groups were significantly decreased compared to the control group (78.3%). All 4N embryos significantly reduced the number of cells in blastocysts compared to control (2N) embryos. The rate of apoptotic cells was not significantly different between the FUTP group and the control group but significantly increased in the CBTP and CITP treatment groups than the control and FUTP groups. In conclusion, our study confirmed that the FUTP method was most effective in preventing apoptosis in the formation of porcine 4N embryos. Further studies are needed to determine whether 4N embryos can support normal piglets of pluripotent cells via 4N complementation.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT: NRF-2021R1C1C2013954).
Keywords: Tetraploid, Developmental competence, Parthenogenetic activation

TOPIC: EPITHELIAL_LUNG

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DIFFERENTIATION OF HUMAN PSC SUSPENSION CULTURES TO DEFINITIVE ENDODERM LINEAGES

Yankaskas, Chris - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Holmes, Spencer - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Scott, Bob - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Kennedy, Mark - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Kuninger, David - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*

Abstract: StemScale PSC Suspension Medium supports the suspension culture of pluripotent stem cells (PSCs) self-assembled into spheroids. Suspension culture simplifies the handling and scale-up of PSC growth and differentiation workflows. Differentiation to the definitive endoderm (DE) – one of the three primary embryonic germ layers – is the first step in generating PSC-derived models of the epithelial cells lining the airway and digestive tract, as well as cells from the lungs, liver, pancreas, thymus, and thyroid. PSC spheroid cultures were differentiated to definitive endoderm cells in suspension using the PSC Definitive Endoderm Induction Kit. DE induction was effective in both human induced-PSCs and human embryonic stem cells. The utility of PSC-derived DE cells was demonstrated by further differentiation in suspension to anterior foregut endoderm cells, and further differentiation to lung epithelial cell organoids. Altogether, PSC suspension culture provides a useful tool for stem cell culture and differentiation.

Keywords: Differentiation, Suspension culture, Definitive Endoderm

TOPIC: GERMLINE

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EFFECT OF MYO-INOSITOL SUPPLEMENTATION ON THE BOAR SEMEN QUALITY DURING THE LIQUID PRESERVATION

Jawad, Ali - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*
Hyun, Sang-Hwan - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Oxidative stress during the liquid preservation of boar semen reduces the motility with the passage of time. However, the addition of the antioxidants showed improved boar sperm motility when stored at 17°C. Myo-inositol is one of the most important antioxidants that improve the human sperm quality and the rate of fertilization. In this study, we investigated the effect of myo-inositol supplementation on the motility and plasma membrane integrity of the boar sperm during liquid preservation. We divided the diluted boar sperm into five groups: a). Control b). 2 mg/mL myo-inositol c). 4 mg/mL myo-inositol d). 6 mg/mL myo-inositol e). 8 mg/mL myo-inositol. In this study, sperm motility was examined on day 1, day 3, and day 5 with CASA. In addition, the plasma membrane integrity of sperm was evaluated via the hypo-osmotic swelling test (HOST) on day 0, day 1, day 3, day 5, and day 7. The results showed that the total motility was increased in the treated groups compared to the control group; however, progressive motility did not show high motility compared with the control group. Moreover, kinematic parameters were improved in the treated groups compared to the control group. The plasma membrane integrity was significantly improved in the treated groups (2mg and 4mg) on the 3rd, 5th, and 7th days of semen storage at 17 °C. In short, myo-inositol supplementation in the groups (2mg/mL, 4 mg/mL) increased the sperm motility and improved the plasma membrane integrity in boar semen during the liquid preservation.

Funding Source: This work was supported by grants from the “NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)” and “IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)”, Republic of Korea
Keywords: Myo-inositol, sperm motility, plasma membrane integrity

TOPIC: HEMATOPOIETIC SYSTEM

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TRANSPOSABLE ELEMENT REGULATION OF HEMATOPOIETIC LINEAGE DECISIONS

Najia, Mohamad - *Hematology/Oncology, Boston Children's Hospital, Cambridge, MA, USA*
Jha, Deepak - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Zhang, Cheng - *Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic College of Medicine and Science, Rochester, MN, USA*
Markel, Arianna - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Li, Hu - *Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic College of Medicine and Science, Rochester, MN, USA*
North, Trista - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Blainey, Paul - *Biological Engineering, MIT, Cambridge, MA, USA*
Daley, George - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*

Abstract: The hematopoietic system is a paradigm of stem cell biology and the focus of decades of inquiry into the molecular wiring that governs differentiation. However, the role of transposable elements (TEs) remains an underexplored layer of genetic regulation. Accumulating evidence suggests TEs have been co-opted as cis regulatory elements that shape gene regulatory networks. Moreover, TEs are finely regulated by cellular epigenetic machinery and thus potently contribute to local chromatin environments. We hypothesized that TEs impart a regulatory architecture through genetic enhancers and chromatin states to guide human hematopoietic fate decisions. We generated a comprehensive atlas of enhancer-gene regulation using the Activity-by-Contact (ABC) model on all major human hematopoietic cell types. Interrogation of 2.5 million enhancer-gene links revealed TEs are disproportionately enriched in ABC enhancers of lymphoid cells compared to other lineages. TE-containing ABC enhancers harbor lineage-specific transcription factor motifs and exhibit cell-type specific enrichments of TE subfamilies. TEs are transcriptionally upregulated during lymphoid differentiation, whereas expression of TE repressive machinery, such as H3K9 methyltransferases and NuRD complex members is reduced, implying that epigenetic regulation of TEs is concomitant with lymphoid fate specification. We systematically perturbed TE regulatory machinery components in umbilical cord blood HSPCs and assessed lymphoid lineage commitment. Knockout of the H3K9 histone methyltransferase EHMT1 resulted in a robust lineage shift from T to NK cells during in vitro differentiation. Further, knockout of TRIM28, an epigenetic co-repressor with well-documented roles in TE repression, phenocopied EHMT1 loss, supporting the conclusion that TE de-repression mediates the lineage shift. NK lineage skewing at the expense of CD19+ B cells was also reproducibly observed in an MS5 stroma assay following EHMT1 or TRIM28 knockout. Functional characterization of resultant NK cells revealed a highly cytotoxic phenotype versus controls. These data underscore the regulatory contributions of TE epigenetic machinery on human hematopoietic lineage decisions and highlights how modulating TEs could be leveraged for hematopoietic cell engineering.

Funding Source: National Science Foundation Graduate Research Fellowship

Keywords: transposable elements, hematopoietic differentiation, natural killer cells

TOPIC: KIDNEY

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SINGLE CELL MULTIOMIC ANALYSIS OF HUMAN KIDNEY ORGANOIDS ELUCIDATES PROXIMAL TUBULE GENE REGULATORY NETWORKS

Yoshimura, Yasuhiro - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Ledru, Nicolas - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Muto, Yoshiharu - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Omachi, Kohei - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Wilson, Parker - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Miner, Jeffrey - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
Humphreys, Benjamin - *Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*

Abstract: During development, changes in chromatin structure regulate gene expression dynamics. A detailed understanding of gene regulatory networks will improve our understanding of organogenesis and disease mechanisms. Previous studies have shown that pluripotent stem cell-derived kidney organoids contain a diverse range of kidney cell types with maturity similar to that of human fetal kidneys. However, the chromatin structure dynamics and gene regulatory changes during nephrogenesis have not been elucidated. We performed single nucleus RNA sequencing (snRNA-seq) and assay for transposase-accessible chromatin with sequencing (snATAC-seq) to investigate human kidney organoid differentiation. We identified cell-type-specific changes in chromatin accessibility and predicted cis-regulatory links between ATAC peaks and target genes. Changes in transcription factor expression were associated with enrichment of their corresponding motifs in ATAC peaks. Kidney organoids were less mature than human adult kidneys as shown by distinct chromatin accessibility patterns in promoter and putative enhancer regions of maturation-related genes. Furthermore, we focused on HNF4A and HNF4G, which are critical transcription factors active in the proximal tubules. By analyzing organoids with either single or double knock-out of these genes and measuring transcription factor occupancy with cleavage under targets and release using nuclease (CUT&RUN) sequencing, we revealed both compensatory and non-overlapping roles for HNF4A and HNF4G in proximal tubule differentiation, especially in the regulation of reabsorption-related genes. In summary, our results demonstrate the importance of gene regulation in cell fate specification and provide insights into cell maturation in human kidney development.

Funding Source: Seed Network Grant CZF2019-002430 from the Chan Zuckerberg Initiative
Keywords: Kidney organoid, Multiomics, CUT&RUN

TOPIC: LIVER

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HUMAN IPSC-DERIVED LIVER ORGANOIDS GENERATED IN ROTATING WALL VESSEL BIOREACTORS DEMONSTRATE IMPROVED DIFFERENTIATED FUNCTIONS COMPARED TO ORGANOIDS PRODUCED ON MATRIGEL

Weng, Yun - *Surgery, University of California, San Francisco, CA, USA*
Han, Simon - *Surgery, University of California, San Francisco, CA, USA*
Sekyi, Maria - *Surgery, University of California, San Francisco, CA, USA*
Su, Tao - *Pathology, University of California, San Francisco, CA, USA*
Mattis, Aras - *Pathology, University of California, San Francisco, CA, USA*
Chang, Tammy - *Surgery, University of California, San Francisco, CA, USA*

Abstract: Human induced pluripotent stem cell (iPSC)-derived liver organoids may be useful tools in disease modeling, drug development, and clinical therapy. Most protocols for generating organoids rely upon Matrigel, an incompletely defined basement membrane matrix extracted from Engelbreth–Holm–Swarm mouse sarcomas. Matrigel's batch-to-batch variability and xenogeneic source pose challenges to organoid research and eventual clinical translation to humans. The rotating wall vessel (RWV) is a low-shear-stress suspension culture system that supports self-assembly of cells into organoids without need of exogenous matrix or scaffolds, and thus may be an alternative to Matrigel-based organoid systems. To determine the differentiation and function of liver organoids generated in RWVs compared to Matrigel, we differentiated human iPSCs into hepatic endoderm, and then induced organoid formation by culturing either on top of Matrigel or within RWVs. Compared to Matrigel organoids, RNA-Seq analysis showed that RWV organoids had significantly higher expression of 256 hepatocyte-specific genes, encompassing a broad spectrum of liver secretory and metabolic functions. Greater functional gene expression in RWV organoids was associated with higher numbers of hepatocyte nuclear factor 4 alpha (HNF4a)-expressing cells and higher expression of cell-cell adhesion molecules. RWV organoids maintained for up to 22 days in culture showed significantly greater albumin secretion, cytochrome P450 1A1 and 3A4 metabolic activity as compared to Matrigel organoids. Our findings indicate that RWV hepatic organoids show greater mature functional characteristics than Matrigel organoids. Matrigel-free organoid self-assembly in RWVs may be a high-throughput way to generate highly functional iPSC-derived organoids for tissue engineering research and therapeutic applications.

Funding Source: This work was supported by NIH R01 DK114311, NASA 16-16ROSBDFP-0030, NSF 1830768, American College of Surgeons Clowes award, and the Open Philanthropy Project.
Keywords: iPSC, liver, organoids

TOPIC: NEURAL

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REDUCE AND REUSE: THE REDISTRIBUTION OF OLFACTORY BULB INTERNEURONS DURING DEVELOPMENT AND THE EVOLUTION OF CELL TYPES IN THE PRIMATE FOREBRAIN

Schmitz, Matthew T. - *Neurology, University of California, San Francisco, CA, USA*
Nowakowski, Tomasz - *Anatomy, University of California ,San Francisco, CA, USA*
Sandoval, Kadellyn - *Biomedical Sciences, University of California, San Francisco, CA, USA*
Paredes, Mercedes - *Neurology, University of California ,San Francisco, CA, USA*
Seeley, Bill - *Neurology, University of California, San Francisco, CA, USA*
Ye, Chun - *Rheumatology, University of California, San Francisco, CA, USA*
Pollen, Alex - *Neurology, University of California, San Francisco, CA, USA*

Abstract: Neuroanatomists have long speculated that expanded primate brains contain an increased morphological diversity of inhibitory neurons and recent studies have identified primate-specific neuronal populations at the molecular level. However, we know little about the developmental mechanisms that specify evolutionarily novel cell types in the brain. Here, we used single cell RNA sequencing to reconstruct gene expression trajectories specifying inhibitory neurons spanning the neurogenic period in human, macaque and mouse. We find that the initial classes of inhibitory neurons generated prenatally are largely conserved among mammals. Nonetheless, we find that multiple classes of transcriptionally-conserved olfactory-bulb bound precursors are redirected to expanded primate white matter and striatum. This includes a novel class of neurons surrounding the striatum, which we call striatum laureatum neurons, that resemble dopaminergic periglomerular cells of the olfactory bulb. In addition, we find that some of the latest-migrating neurons from the neural stem cell niche traverse a novel migratory stream in primates into the cortical white matter instead of the olfactory bulb. We then examined spatio-temporal gene expression divergence between cortical- and olfactory bulb-bound neurons. Through this, we propose an evolutionary model in which conserved initial classes of neurons supplying the smaller primate olfactory bulb are reused in the enlarged striatum and cortex.

Funding Source: F31NS124333 (M.T.S), DP2MH122400-01 (A.A.P), U01MH114825 (A.A.P, T.JN), R01AI136972 (C.J.Y), DP2NS122550-01 (M.F.P.)
Keywords: Neural Stem Cells, Brain Evolution, Adult Neurogenesis

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GENETIC POLYMORPHISMS AND CLINICAL IMPLICATION IN BLEPHAROSPASM

Jang, Jeong-Kyeong - *Department of Ophthalmology, CHA University / Bundang CHA Medical Center, Seongnam, Korea*
Kwon, Min Jung - *Department of Biomedical Science, CHA University, Seongnam, Korea*
Kim, Nam Keun - *Department of Biomedical Science, CHA University, Seongnam, Korea*

Lew, Helen - *Department of Ophthalmology, CHA Bundang Medical Center, Seongnam, Korea*

Abstract: Blepharospasm (BSP) is the focal dystonia involving periocular muscles and result in involuntary forceful eyelid closure and functional blindness. Some studies reported that familial dyskinesia with facial myokymia (FDFM) is related to mutation in Adenylyl cyclase 5 (ADCY5) but other study presented no association of genetic variant which related to vascular compression in hemifacial spasm (HFS). As the pathogenesis of BSP is not completely understood, we have recently demonstrated that crucial roles of single nucleotide polymorphisms (SNPs) in BSP patients. We established a prospective case-controlled study including 49 patients with BSP and 197 controls in South Korea. Blood samples were collected and then centrifuged to extract genomic DNG (gDNA). Human gDNA was analyzed by polymerase chain reaction based restriction fragment length polymorphism (PCT-RNFL) assay and real-time PCR with TaqMan probe to confirm the frequency of the following polymorphisms: TOR1A rs1801968C>G, rs1182C>A, DRD2 rs1800497G>A, DRD5 rs6283C>T which related to neurological signaling pathway. We found DRD2 rs1800497 mutant was significantly associated with BSP risk (Odds ratio; OR = 0.338, Confidential interval; CI = 0.115-0.995, p = 0.049) and also improved ability of DRD2 rs1800497 mutant which adjusted with age and sex (Adjusted odds ratio; AOR = 0.329, CI = 0.111-0.977, p = 0.045). We also found genetic susceptibility of DRD5 rs6283 CC genotype in BSP (OR = 0.270, CI = 0.076-0.965, p = 0.044), and significant association of DRD5 rs6283 mutant with BSP ocular form (AOR = 0.385, CI = 0.172-0.862, p = 0.020). We demonstrated the genetic polymorphisms in BSP patients compared to age-controlled group. Based on our findings, the genetic polymorphisms combined with the neurological signaling pathway would be expected to elucidate a foundation for the genetic factors in BSP, HFS, and other facial myokymia.

Keywords: Blepharospasm, Genotype analysis, Polymorphism

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RAPID DETECTION OF PALLIAL GABAERGIC INTERNEURON SUBTYPES BY SINGLE NUCLEI RNA SEQUENCING AFTER PRECLINICAL TRANSPLANTATION OF NRTX-1001, A CELLULAR THERAPEUTIC IN CLINICAL DEVELOPMENT FOR EPILEPSY

Bershteyn, Marina - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Zhou, Hongjun - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Fuentealba, Luis - *Preclinical, Neurona Therapeutics, South San Francisco, CA, USA*
Subramanyam, Geetha - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Sezan, Meliz - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Cherkowsky, Daniel - *Preclinical, Neurona Therapeutics, South San Francisco, CA, USA*
Bates, Adrian - *Preclinical, Neurona Therapeutics, South San Francisco, CA, USA*
Havlicek, Steven - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Maury, Yves - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*
Bulfone, Alessandro - *Operations, Neurona Therapeutics, South San Francisco, CA, USA*
Banik, Gautam - *Process Sciences, Neurona Therapeutics, South San Francisco, CA, USA*
Priest, Catherine - *Preclinical, Neurona Therapeutics, South San Francisco, CA, USA*
Nicholas, Cory - *Discovery Research, Neurona Therapeutics, South San Francisco, CA, USA*

Abstract: Inhibitory neuron cell therapy represents a promising therapeutic strategy for chronic neurological disorders characterized by local neural circuit hyperexcitability. We hypothesized that a specific lineage of GABAergic neurons is required for optimal efficacy in suppressing seizures for the treatment of epilepsy: namely, pallial interneurons derived from progenitor cells in the medial ganglionic eminence (MGE) and characterized by the expression of somatostatin (SST) and parvalbumin (PV). However, MGE progenitors give rise to a variety of other lineages beside pallial interneurons, including subpallial GABAergic projection neurons, cholinergic neurons and glial cells. Also, neighboring lateral and caudal ganglionic eminence progenitors generate different types of neurons. In a therapeutic setting, these cell populations could be considered “off-target”. Thus, careful characterization of graft composition and cell fate post-transplantation is essential, albeit challenging, given the protracted development of human neurons. We have developed NRTX-1001, a novel hPSC-derived GABAergic interneuron cell therapeutic candidate, which was recently cleared by the FDA for a first-in-human phase I/II clinical trial in people with drug-resistant mesial temporal lobe epilepsy. Extensive characterization of product composition using functional and single cell RNAseq assays demonstrated consistent generation of migratory, post-mitotic MGE-type GABAergic neurons of a pallial-like interneuron lineage. We then investigated graft composition following transplantation of the human MGE-type interneurons into the rodent brain using single nuclei RNA-seq. Remarkably, despite the protracted maturation of human interneurons, bioinformatics analyses were able to predict subtype fate just one month after transplantation based on global gene expression patterns and revealed that the human grafts consist of GABAergic pallial interneuron sublineages expressing SST, PV or SST/NPY. Collectively, these findings demonstrate that the transplantation of hPSC-derived MGE-type GABAergic neurons can consistently generate appropriate pallial interneuron subtypes and pave the way for understanding how subtype composition impacts the efficacy of interneuron cell therapy.

Keywords: GABAergic interneurons, Epilepsy, Cell therapy

TOPIC: PANCREAS

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THE INITIAL SEEDING CONCENTRATION AND DIAMETER OF PLURIPOTENT STEM CELL AGGREGATES IMPACT THE GENERATION OF PANCREATIC PROGENITORS

Iworima, Diepiriye G. - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Baker, Robert - *Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada*
Piret, James - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Kieffer, Timothy - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*

Abstract: Human pluripotent stem cells (hPSCs) can be used to generate both pancreatic progenitors and endocrine cell types. These PSC-derived cells have been shown to prevent or reverse chemically-induced diabetes in rodent models. Different cell culture strategies are employed to generate both pancreatic progenitors and endocrine cells. These strategies include monolayer, suspension, or a hybrid monolayer-suspension method of cell culture during the differentiation. We previously demonstrated based on the expression of key transcription factors PDX1 and NKX6.1 that the initial seeding density on an adherent monolayer can impact the differentiation efficiency towards pancreatic progenitors (stage 4). In this study, we tested initial seeding concentrations from 0.25 x 106 cells/mL to 1.5 x 106 cells/mL. We also generated hPSC clusters with average diameters of either 77 µm or 130 µm, in vertical wheel bioreactors prior to differentiation. The definitive endoderm stage (stage 1) was successfully induced in all conditions with an average of 99% FOXA2+/SOX17+ based on flow cytometry. Low levels of hypoxia were observed at the end of stage 1. As early as the primitive gut stage (stage 2), there were obvious aggregate morphology differences, such as variable degrees of loose cells within clusters with a distinct "balloon" phenotype observed in some conditions. By the end of stage 4, morphologies ranged between hollow clusters, blastocyst-like clusters and compacted aggregates. Furthermore, there was a net increase in aggregate size, with mean diameters between 170 and 200 µm. For the conditions tested, the net yield of cells by the end of stage 4 ranged from 1% to 300% of the input hPSC, with cell viability over 90% in most conditions. The number of pancreatic progenitors generated, based on flow cytometry measurements of the PDX1+/NKX6.1+ population, was between 29% - 90%. Gene expression data for NKX6.1 and PDX1 using RT-qPCR trended similarly to the protein levels observed by flow cytometry. Our results based on NKX6.1 and PDX1 suggest that initial seeding concentration and aggregate size are critical process parameters that affect the quality attributes of PSC-derived pancreatic progenitors.

Keywords: pancreatic progenitors, stem cell aggregates, bioprocess development

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EFFECTS OF OXYGEN ON THE DIFFERENTIATION OF STEM CELL-DERIVED PANCREATIC BETA CELLS

Jennings, Lauren - *Department of Physiology and Biomedical Engineering, Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN, USA*
Peterson, Quinn - *Department of Physiology and Biomedical Engineering, Center for Regenerative Medicine, Mayo Clinic, Rochester, MN, USA*

Abstract: The loss of insulin-secreting pancreatic beta cells results in elevated blood glucose levels in people with type 1 diabetes (T1D), a disease that affects millions worldwide. The study of disease progression, identification of drug targets, and development of cellular therapies for T1D are inhibited by the limited availability of human pancreatic tissue. The recent development of directed differentiation protocols for the generation of stem cell-derived beta cells (SC-β) paves the way for the development of new cell-based therapeutics for T1D. SC-β cells express markers of mature beta cells, mimic physiological responses to metabolic stimuli, and secrete insulin in response to varying glucose levels. Despite this advancement, current protocols for the generation of SC-β cells are fraught with variability and often result in functionally immature cell products. Thus, optimized methods to consistently manufacture high-quality SC-β cells are needed. Efforts toward generating SC-β cells have been based on stirred bioreactors in tissue culture incubators that lack precise oxygen control. Here, we demonstrate that controlling the exposure to oxygen during the differentiation process provides an opportunity to improve the differentiation of SC-β cells by more closely recapitulating the natural environment of development. Cells exposed to altered oxygen levels at precise stages of differentiation exhibit changes in differentiation capacity, cell-cell connectivity, and metabolic activity. Additionally, changes in the expression of key developmental markers and oxygen sensing pathways were observed, confirming that differentiating human embryonic stem cells are responsive to varying oxygen conditions. Modulating oxygen levels affected the progression of cells through the stepwise differentiation process towards SC-β cells, and optimal oxygen conditions were identified. Improving the understanding of how oxygen conditions affect the differentiation of SC-β cells can lead to the production of better cells for the treatment of T1D, as well as in vitro disease modeling and drug screening.

Keywords: beta cell, diabetes, oxygen

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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REGIONALLY DISTINCT TROPHOBLAST REGULATE BARRIER FUNCTION AND INVASION IN THE HUMAN PLACENTA

Marsh, Bryan - *Developmental and Stem Cell Biology, University of California, San Francisco, CA, USA*
Zhou, Yan - *Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA*
Kapidzic, Mirhan - *Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA*
Fisher, Susan - *Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA, USA*
Blelloch, Robert - *Urology, UCSF, San Francisco, CA, USA*

Abstract: The human placenta forms the essential bridge between maternal tissues and the developing fetus. Placental development occurs asymmetrically resulting in two specialized regions. One forms branched villi which anchor into the maternal decidua allowing extra-villous trophoblasts (EVTs) to invade deep into maternal tissue. This region is essential for the exchange of factors between the mother and the fetus. The other region forms a stratified epithelium lacking villi and invasive cells but that surrounds more than 70% of the chorionic sac. Termed the smooth chorion, its cellular composition and function is relatively unknown. Here, we use single cell sequencing to compare the cellular states and molecular architecture of the villous and smooth chorion regions of the human placenta. Cell states and trajectories are similar between regions with one striking exception. In the villous region, cytotrophoblasts (CTB) differentiate into syncytiotrophoblasts and EVTs. In contrast, transcriptionally similar CTB in the smooth chorion differentiate into a smooth chorion specific CTB population (SC-CTB). SC-CTB cells express a distinct epidermal and metabolic transcriptional program consistent with a barrier-like function. Surprisingly, EVTs in the smooth chorion and villous regions appear transcriptionally similar despite differences in the depth of invasion. EVT remain within the stratified epithelium in contact with SC-CTB cells in the smooth chorion, but physically separate

from all CTB cells in the villous region. This juxtaposition suggests that SC-CTB might inhibit migration of EVTs in the smooth chorion. Consistent with this hypothesis, conditioned media from cells of the smooth chorion restricts invasion of EVTs from the villous chorion region in an in vitro invasion assay. Computational prediction of potential ligand-receptor interactions identifies cytokines released from the SC-CTB as potential regulators, which are now being tested in functional assays. These data identify a novel CTB population specific to the smooth chorion of the human placenta that forms an epidermal-like barrier and limits the invasion of EVTs specifically to the villous region. These new insights have broad implications for the molecular mechanisms of placental development and treatment of prenatal diseases.

Funding Source: NICHD 2P50HD055764
Keywords: Placenta, Cell Fate Specification, Development

TOPIC: PLURIPOTENT STEM CELLS

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THE IMPACT OF RECURRENT CHROMOSOMAL ABNORMALITIES ON GROWTH ADVANTAGE AND DIFFERENTIATION CAPACITY OF HUMAN PLURIPOTENT STEM CELLS

Krivec, Nuša - *Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium*
Couvreu de Deckersberg, Edouard - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Lei, Yingnan - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Sermon, Karen - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Spits, Claudia - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Verhulst, Stefaan - *Liver Cell Biology, Vrije Universiteit Brussel, Belgium*

Abstract: Human pluripotent stem cells can differentiate to any cell type in the adult human body, which makes them a promising tool for disease modeling and transplantation. However, genetic abnormalities can arise in in vitro culture and grant the cells a selective advantage, two of the most frequently identified being gains of chromosomes 1 and 20. Currently, the exact effect of most recurrent abnormalities on lineage specification is not yet understood. The aims of this research are to study if cells with recurrent genetic abnormalities retain their in vitro culture advantage during differentiation and how these abnormalities affect their differentiation efficiency to cell types of three germ layers. For this, we created mosaic cultures by mixing 10% of fluorescently labelled mutant cells with 90% of their karyotypically normal isogenic counterparts. The mixes were differentiated in triplicate to hepatocyte, cardiomyocyte and neuroectoderm progenitors. The ratio of mutant and wild type cells was determined before and after differentiation with flow cytometry. The differentiation efficiency was determined with immunostaining and qPCR for specific differentiation markers. We found that selective advantage of mutant cells is maintained during differentiation to neuroectoderm and hepatocyte progenitors. The population of cells with a 1q duplication increased from 8% to 58% during neuroectoderm differentiation and from 24% to 87% during hepatoblast differentiation. A similar increase in number of mutant cells was observed in cells with 20q11.21 duplication. Both 1q and 20q cells successfully exit the pluripotent state but fail to equally commit to neuroectoderm and hepatoblast linage as their genetically balanced isogenic counterparts. The experiments on cardiomyocytes are ongoing. Our work reveals for the first time that the selective advantage of chromosomal abnormalities is retained during differentiation, showing that having a culture that is mosaic at the beginning of the differentiation can result in take-over of aneuploid cells during the differentiation and a heterogeneous end population of poorly specified cells.

Funding Source: The Research Foundation – Flanders (FWO)
Keywords: Human embryonic stem cells, Genetic abnormalities, Growth advantage during differentiation

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PRINCIPLES OF STRESS GRANULE FORMATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS

Emara, Mohamed - *Basic Medical Sciences, QU Health, Qatar University, Doha, Qatar*
Abdulla, Sara - *Neurological Disorders Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar*
Engelke, Rudolf - *Proteomics Core, Weill Cornell Medical College-Qatar, Doha, Qatar*
Mousa, Hanaa - *Basic Medical Sciences Department, College of Medicine, QU Health, Qatar University, Doha, Qatar*
Palangi, Freshteh - *Neurological Disorders Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar*
Salloum-Asfar, Salam - *Neurological Disorders Research Center, Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Doha, Qatar*
Schmidt, Frank - *Proteomics Core, Weill Cornell Medical College-Qatar, Doha, Qatar*

Abstract: Stress Granules (SGs) are dynamic ribonucleoprotein aggregates, which have been observed in cells subjected to environmental stresses. Pluripotent stem cells (PSCs) are highly sensitive to different stresses, indicating the importance of SGs in regulating stem cell fate. Therefore, our work aims to establish whether these granules have a role in regulating PSC self-renewal and differentiation. We compared the effects of different types of stresses on SG formation in human induced (hi) PSCs. We found that oxidative, thermal, and hyperosmotic stresses induce SG formation in hiPSCs. The analyses of these granules showed that they are canonical SGs, because (i) they contain the well-known SGs proteins (G3BP, TIAR, eIF4E, eIF4A, eIF3B, eIF4G, and PABP), (ii) they were found in juxtaposition to processing bodies (PBs), and (iii) they were disassembled after the removal of the stress. Moreover, Oxidative and thermal stresses promote eIF2α phosphorylation in hiPSCs forming SGs. Interestingly, gradient concentrations of hyperosmotic stresses showed a specific pattern of SG formation in hiPSCs, which is different than other stresses. Moreover, the formation of hyperosmotic SGs in hiPSCs was independent of eIF2α phosphorylation and was associated with low apoptosis levels. Since hyperosmotic stresses showed this specific pattern of SG formation in hiPSCs, we performed comprehensive proteomics analysis to identify proteins involved in regulating such pattern. We found possible implications of microtubule organization in response to hyperosmotic stress in hiPSCs. We have also unveiled a reduced expression of tubulin that may protect cells against hyperosmolarity stress while inhibiting SG formation without affecting the stem cell self-renewal and pluripotency. Altogether, our data introduce new aspects and possible

mechanisms of how hiPSCs respond to adverse environmental conditions.

Funding Source: This study was supported by QBRI internal grant (QB16) and the Qatar University Student grant (QUST-2-CMED-2019-1).
Keywords: Stress granules, Pluripotent Stem cell, Microtubules

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PLAKOGLOBIN IS A MECHANORESPONSIVE REGULATOR OF NAÏVE PLURIPOTENCY

Kohler, Timo N. - *Biochemistry, University of Cambridge, UK*
De Jonghe, Joachim - *Francis Crick Institute, Francis Crick Institute, London, UK*
Ellermann, Anna - *Biochemistry, University of Cambridge, UK*
Yanagida, Ayaka - *Division of Stem Cell Therapy, University of Tokyo, Japan*
Herger, Michael - *Biochemistry, University of Cambridge, UK*
Slatery, Erin - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Fischer, Katrin - *Biochemistry, University of Cambridge, UK*
Mulas, Carla - *Randall Centre for Cell and Molecular Biophysics, King's College London, London, UK*
Winkel, Alex - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Ross, Connor - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Bergmann, Sophie - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Franze, Kristian - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Chalut, Kevin - *Physics, University of Cambridge, UK*
Nichols, Jennifer - *MRC Human Genetics Unit, University of Edinburgh, UK*
Boroviak, Thorsten - *Physiology, Development and Neuroscience, University of Cambridge, UK*
Hollfelder, Florian - *Biochemistry, University of Cambridge, UK*

Abstract: Biomechanical cues are instrumental in guiding embryonic development and cell differentiation. Understanding how these physical stimuli translate into transcriptional programs could provide insight into mechanisms underlying mammalian pre implantation development. Here, we explore this by exerting microenvironmental control over mouse embryonic stem cells (ESCs). Microfluidic encapsulation of ESCs in agarose microgels stabilized the naïve pluripotency network and specifically induced expression of Plakoglobin (Jup), a vertebrate homologue of beta-catenin. Indeed, overexpression of Plakoglobin was sufficient to fully re establish the naïve pluripotency gene regulatory network under metastable pluripotency conditions, as confirmed by single cell transcriptome profiling. Finally, we found that in the epiblast, Plakoglobin was exclusively expressed at the blastocyst stage in human and mouse embryos – further strengthening the link between Plakoglobin and naïve pluripotency in vivo. Our work reveals Plakoglobin as a mechanosensitive regulator of naïve pluripotency and provides a paradigm to interrogate the effects of volumetric confinement on cell fate transitions.

Funding Source: Dr. Timo N. Kohler received a fully funded PhD scholarship from AstraZeneca.
Keywords: Pluripotency, Development, Microfluidics

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INTEGRATIVE MULTI-OMICS REVEALS DISTINCT EPIGENETIC STATES FOR DIFFERENT STAGES OF HUMAN PLURIPOTENCY

Zijlmans, Dick W. - *Molecular Biology, Radboud University Nijmegen, Netherlands*
Talon, Irene - *Development and Regeneration, KU Leuven, Belgium*
Verhelst, Sigrid - *Department of Pharmaceutics, Ghent University, Ghent, Belgium*
Bendall, Adam - *Epigenetics, Babraham Institute, Cambridge, UK*
Malcolm, Andrew - *Epigenetics, Babraham Institute, Cambridge, UK*
Biggins, Laura - *Bioinformatics, Babraham Institute, Cambridge, UK*
To, San Kit - *Development and Regeneration, KU Leuven, Belgium*
Janiszewski, Adrian - *Department of Pharmaceutics, KU Leuven, Belgium*
Admiraal, Danielle - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*
Knops, Ruth - *Hematology, Radboud UMC, Nijmegen, Netherlands*
Bhanu, Natarajan - *Biochemistry and Biophysics, University of Philadelphia, PA, USA*
Collier, Amanda - *Epigenetics, Babraham Institute, Cambridge, UK*
Fabian, Charlene - *Epigenetics, Babraham Institute, Cambridge, UK*
Allsop, Ryan N. - *Development and Regeneration, KU Leuven, Belgium*
Garcia, Benjamin - *Biochemistry and Biophysics, University of Philadelphia, PA, USA*
Vermeulen, Michiel - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*
Dhaenens, Maarten - *Department of Pharmaceutics, Ghent University, Ghent, Belgium*
Marks, Hendrik - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*
Rugg-Gunn, Peter - *Epigenetics, Babraham Institute, Cambridge, UK*
Pasque, Vincent - *Development and Regeneration, KU Leuven, Belgium*

Abstract: During the course of development, the human embryo gives rise to distinct pluripotent stem cells (PSCs), which can be modelled in vitro. Naïve PSCs represent the pre-implantation epiblast, whereas primed PSCs represent the post-implantation epiblast. Whilst the lineage potential of naïve PSCs is considered to be unrestricted, primed PSCs appear to be developmentally more advanced. In human, this is evidenced by the remarkable ability of naïve PSCs to form trophectoderm, in contrast to primed cells. It has become increasingly clear that naïve and primed PSCs exhibit distinct epigenetic states, affecting their developmental potential. The characterization of these epigenetic states will advance our understanding of naïve-to-primed transition in the embryo and provide insight into what governs stem cell commitment. Here, we define the chromatin-associated proteome, histone post-translational modifications (hPTM) and transcriptome of human naive and primed pluripotent stem cells. Our integrative analysis reveals differences in the relative abundance and activities of distinct chromatin modules. We show an unexpected, remarkable decoupling of the presence of epigenetic writers and

erasers on the chromatin and the respective hPTM landscape between naïve and primed hPSCs. In addition, we observe that chemical inhibition of the epigenetic regulatory module Polycomb Repressive Complex 2 (PRC2) alters the hPTM landscape of naïve hPSCs such that it adopts primed-like features, highlighting the role of the epigenome in maintaining stable stem cell states. Altogether, this work provides a comprehensive characterization of the epigenome of two distinct states of pluripotency, helping us better understand the difference in developmental potential between pluripotent states.

Funding Source: This work is supported by the FWO, KU Leuven, BBSRC, MRC, the Wellcome Trust, NWO-XS, Dutch Cancer Society and NIH.
Keywords: Naive pluripotency, Polycomb Repressive Complex 2, Multi-omics

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IDENTIFICATION OF NOVEL FACTORS ORCHESTRATING EPIBLAST DEVELOPMENT VIA A FULLY AUTOMATED LARGE-SCALE FUNCTIONAL SCREEN

Chen, Fei - *Embryonic Self-Organization Group, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Bruder, Jan - *Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Wu, Jie - *Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Switzerland*
Koesters, Manuel - *Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Switzerland*
Stehling, Martin - *Flow Cytometry Unit, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Drexler, Hannes - *Mass Spectrometry Unit, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Fan, Rui - *Embryonic Self-Organization Group, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Schöler, Hans - *Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany*
Leidel, Sebastian - *Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Switzerland*
Bedzhov, Ivan - *Embryonic Self-Organization Group, Max Planck Institute for Molecular Biomedicine, Münster, Germany*

Abstract: During the peri-implantation stage, the pluripotent epiblast is reshaped from a simple ball of cells into a polarized cup-shaped epithelium. At the same time, the pre-implantation (naïve) pluripotent state is dismantled and transforms into formative (early post-implantation) pluripotency. As the peri-implantation embryos are relatively inaccessible and provide limited material for analysis, the intrinsic signalling mechanisms that coordinate the epiblast morphogenesis and the pluripotency states transition are largely unexplored. Therefore, we used a three dimensional (3D) embryonic stem cell (ESCs) based model of epiblast development to examine this process. We established an automated pipeline for ESCs culture, microscopy and image analysis, which allowed us to massively scale up the 3D culture system into a multi-well format. Taking advantage of this pipeline, we performed a large-scale functional screen using pharmacological libraries containing over 1800 previously validated inhibitors of developmental, metabolic and epigenetic factors. Among the known regulators, we found several novel factors that control the self-organisation and exit of naive pluripotency. In particular, suppressing the activity of protein kinase X (PKX) resulted in a failure of epithelialisation and substantial delay in the transition to formative state. This effect was further enhanced by combining Mek1/2 and PKX inhibition, which also allowed the de novo derivation of ESCs from mouse blastocysts. Investigating the molecular mechanism of PKX function in ESCs revealed that PKX forms a complex with Erk, enabling Erk phosphorylation in a Mek1/2-independent manner. Thus, this newly discovered interaction indicates that cell-intrinsic pro-differentiation cues underline the transient nature of the pluripotent states in the developing embryo.

Keywords: pluripotent epiblast cells, self-organisation, automated 3D culture

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DPPA2 AND DPPA4 MAINTAIN ROBUSTNESS OF POISED CHROMATIN IN HUMAN PLURIPOTENT STEM CELLS

Malcolm, Andrew A. - *Epigenetics, Babraham Institute, Cambridge, UK*
Bendall, Adam - *Epigenetics, Babraham Institute, Cambridge, UK*
Collier, Amanda - *Biological Chemistry, University of California, Los Angeles, CA, USA*
Bevan, Stephen - *Epigenetics, Babraham Institute, Babraham, UK*
Fabian, Charlene - *Epigenetics, Babraham Institute, Babraham, UK*
Semprich, Claudia - *Epigenetics, Babraham Institute, Babraham, UK*
Reik, Wolf - *Epigenetics, Babraham Institute, Babraham, UK*
Rugg-Gunn, Peter - *Epigenetics, Babraham Institute, Babraham, UK*

Abstract: Precise co-ordination of cell fate specification during human early development is a vital yet poorly understood process. To navigate the dynamic transcriptional and epigenetic changes associated with germ layer allocation, pluripotent cells maintain developmentally important genes and their regulatory regions in a poised but repressed chromatin state. The poised state allows for precise and coordinated activation or complete repression of gene expression dependent on instructive signals from the external and intrinsic environment. Poised promoters and enhancers adopt multivalent histone modification states, comprised of both active and repressive modifications, such as H3K27me3, H3K4me3 and H3K4me1. Combining opposing modifications may maintain robustness of genes to low levels of signal whilst retaining the capacity to respond upon the appropriate level. Despite their importance in controlling cell fate decisions, our understanding of the mechanisms by which poised states are established and maintained in human cells is currently lacking. Here, we establish a role for the transcription factors DPPA2 and DPPA4 in maintaining poised chromatin in human pluripotent stem cells (hPSCs). We find that DPPA2/4 bind to the majority of poised promoters and a subset of poised enhancers. CRISPR-Cas9 mediated knockout of DPPA2/4 in primed hPSCs led to depletion of H3K27me3 at a subset of DPPA2/4-target regions, including at the promoters of developmental regulators. Consequently, we detect changes in the expression of these genes, particularly those associated with developmental signalling. Primed hPSCs lacking DPPA2/4 exhibit increased spontaneous differentiation even in self-renewing conditions, display altered cell fate commitment during differentiation and show axial patterning defects upon human gastruloid formation. These data reveal a role for DPPA2/4 in safeguarding the robustness of poised chromatin states and in regulating genes that are important for cell fate specification. Understanding fur-

ther how these transcription factors and chromatin states jointly contribute to gene expression dynamics during development is critical to uncovers the principles of developmental gene regulation and to improve generation of specialised cell types from human pluripotent cells.

Funding Source: Andrew Malcolm is the recipient of a Wellcome Trust four-year PhD studentship.
Keywords: DPPA2/4, Epigenetic Regulation, Poised chromatin

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CRISPR SCREEN TO IDENTIFY BAF SUBUNITS REQUIRED FOR STEM CELL MAINTENANCE AND PLURIPOTENCY

McCubbin, Ryan - *Biology, San Diego State University, San Diego, CA, USA*
Gao, Fangjian - *MCBL-A, Salk Institute for Biological Studies, San Diego, CA, USA*
Hargreaves, Diana - *MCBL-A, Salk Institute for Biological Studies, San Diego, CA, USA*

Abstract: Stem cell pluripotency and lineage specification rely on tightly regulated changes in gene expression. Chromatin remodeling complexes facilitate dynamic rearranging of genomic architecture necessary for enhancer accessibility and transcription factor binding. BAF complexes are multimeric chromatin remodelers that hydrolyze ATP to alter chromatin structure, defining the epigenetic landscape and transcriptome through activation or repression of genomic targets. Consisting of 15 interchangeable subunits out of 29 total subunits encoded in the mammalian genome, BAF complexes possess diverse regulatory roles owing to hundreds of possible combinatorial assemblies. Tissue-specific and cell-specific subunit compositions regulate distinct cellular physiological processes. In embryonic stem cells (ESCs), a stage-specific subunit composition (esBAF) maintains properties such as pluripotency and lineage specification. Although esBAF composition is well-characterized, the contribution of specific subunits in maintaining pluripotency and germ layer specification is not well understood. CRISPR-Cas9 is a powerful tool for high-throughput screening and functional characterization of specific genetic elements associated with a phenotype. In this study, we used CRISPR-mediated genetic knockouts to perturb function of individual subunits in human ESCs (hESCs) and assessed their roles through negative phenotype selection. Oct4-GFP hESC line was used to identify subunits required for self-renewal and RUES-GLR line with fluorescent-labeled lineage-specific genes was used to identify subunits required for pluripotency. hESCs retaining stem cell-specific Oct4-GFP expression or ability to differentiate into each germ layer after in vitro differentiation were obtained through FACS. Genome-integrated sgRNA from hESCs before and after sorting was amplified and subjected to Next-Generation Sequencing to determine which subunit knockouts hindered maintenance of self-renewal and pluripotency. Because BAF subunit composition is a critical determinant for cell identity, a plethora of cancers and developmental disorders arise from subunit mutations. Deeper insight into specific BAF subunit roles will help elucidate mechanisms underlying pathogenesis of various human diseases.

Keywords: Pluripotency, Epigenetics, CRISPR

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CAUGHT IN THE MIDDLE: LEVERAGING THE UNIQUE PROPERTIES OF INTERMEDIATE PLURIPOTENT STEM CELLS TO STUDY FORMATIVE PLURIPOTENCY AND GERMLINE SPECIFICATION

Ballard, Emily D. - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Yu, Leqian - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Wei, Yulei - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Wu, Jun - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Abstract: Pluripotency, or the ability to contribute to all three germ layers and the germline, is a transient property of the epiblast cells during early development. It can be approximated in vitro by culturing epiblast cells from different developmental stages in media that prevent them from differentiation. We and others have recently stabilized in vitro an intermediate phase of pluripotency that lies between the two conventional naive and primed states. This intermediate state, also known as the formative state, is functionally characterized by dual competence for chimera formation in vivo and direct responsiveness to primordial germ cell-like cell (PGC-LC) induction in vitro. The formative-like cells we generated, termed FTW-PSCs, are cultured in a condition containing factors that activate the FGF, TGFβ, and WNT signaling pathways. FTW-PSCs share features of both naive and primed PSCs, such as their lack of X inactivation and germline chimera competence (naive), and formation of tight junctions and higher levels of histone methylation (primed). Leveraging the unique properties of FTW-PSCs, we are now focused on identifying key regulators of formative pluripotency. Through loss-of-function studies, we have recently confirmed the roles of several putative formative transcription factors (TFs), including Otx2, Oct6, and Sox3, in the maintenance of mouse FTW-PSCs. We are also investigating the transcriptional, epigenetic, and functional differences between PGC-LCs derived from FTW-PSCs and transient formative EpiLCs (epiblast-like cells) induced from naïve PSCs. For future studies, we plan to perform ChIP-seq and CAPTURE-proteomics to identify novel upstream regulators and downstream targets of formative TFs. With this approach, we hope to uncover the network of genes underlying formative pluripotency and further characterize the germline competency of these cells.

Keywords: pluripotent stem cells, formative pluripotency, primordial germ cells

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AKT-TORS IN DIFFERENTIATION: THE ROLE OF PTEN AND TSC2 IN COORDINATING ES CELL EXIT FROM NAÏVE PLURIPOTENCY

Santini, Laura - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs, University of Vienna, Austria*
Titz-Teixeira, Fabian - *Faculty of Mathematics and Natural Sciences, CECAD, University of Cologne, Germany*
Beyer, Andreas - *Faculty of Mathematics and Natural Sciences, CECAD, University of Cologne, Germany*
Leeb, Martin - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs, University of Vienna, Austria*

Abstract: Mouse embryonic stem cells (mouse ES cells) are captured in what is referred to as the ground state of pluripotency. This cell state, also known as the naïve pluripotent state, is sustained by a self-reinforcing gene regulatory network (GRN) composed by core pluripotency transcription factors (TFs) and naïve-specific TFs. Upon appropriate instructions, ES cells exit the naïve pluripotent state and enter the differentiation process, transitioning through the recently described formative pluripotent state. This is accompanied by the dismantling of the naïve GRN, and the establishment of a formative-specific GRN. A balanced interplay of several signalling pathways regulates this cell state transition. Among those, an important role in supporting the self-renewing, naïve pluripotent state of ES cells is exerted by the PI3K/Akt pathway. Two negative regulators of this pathway, Pten and Tsc2, are indeed found among high confidence hits in genetic screens performed to identify factors driving ES cell differentiation. Although Pten and Tsc2 are already reported to be involved in the control of ES cell exit from naïve pluripotency, the molecular mechanisms underlying their differentiation promoting function remains to be addressed. Combining the use of knock-out (KO) cell lines with small molecules inhibitors, we confirmed the requirement of Pten and Tsc2 for proper ES cell differentiation. Mechanistically, we observed that TSC2, as expected from textbook knowledge, controls ES cell differentiation via mTORC1 signalling. PTEN signal is mainly, but not completely, integrated through mTORC1. Several observations point to a parallel involvement of FoxO signalling downstream PTEN. Conversely, the GSK3 pathway seems to play only a minor role in integrating PTEN signalling. Our ultimate goal is the identification of common and specific downstream targets of the signalling cascades elicited by PTEN and TSC2. This will provide a better understanding of molecular events orchestrating the early differentiation process of ES cells, clarifying the functions exerted by negative regulators of the PI3K/Akt pathway in coordinating the exit from the naïve pluripotent state, and the entry into the formative pluripotent state.

Keywords: Pluripotency, Signalling pathways, Cell state

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A GENOME-WIDE CRISPR SCREEN TO IDENTIFY NOVEL PATHWAYS INVOLVED IN REPROGRAMMING AND X-CHROMOSOME REACTIVATION

Barrero, Mercedes - *Gene Regulation, Stem Cells and Cancer, Centre for Genomic Regulation, Barcelona, Spain*
Blanco, Enrique - *Gene Regulation, Stem Cells and Cancer, Centre for Genomic Regulation, Barcelona, Spain*
Bauer, Moritz - *Gene Regulation, Stem Cells and Cancer, Centre for Genomic Regulation, Barcelona, Spain*
di Croce, Luciano - *Gene Regulation, Stem Cells and Cancer, Center for Genomic Regulation, Barcelona, Spain*
Payer, Bernhard - *Gene Regulation, Stem Cells and Cancer, Centre for Genomic Regulation, Barcelona, Spain*

Abstract: Species with genetic sex determination present differences in chromosome composition between the sexes. In mammals, male cells contain one X and one Y chromosome, while female cells have two X chromosomes. In order to achieve an equal sex-linked gene dosage, female cells silence one X chromosome in a process called X-chromosome inactivation (XCI). The silent state of the inactive X chromosome is stably maintained in somatic cells. However, XCI is reversed during development by a process called X-chromosome reactivation (XCR), which takes place in the pluripotent epiblast of the blastocyst and in the germ cell lineage. In the laboratory, XCR can also be studied using in vitro systems, like reprogramming of female differentiated somatic cells to induced pluripotent stem cells (iPSCs). Although previous studies have undertaken candidate approaches, the regulatory network involved in XCR during reprogramming has not been fully investigated. Therefore, we performed an unbiased genome-wide pooled CRISPR-screening approach to identify factors and pathways involved in XCR. To this end, taking advantage of an X-chromosome reporter cell line, we reprogrammed mouse neural precursor cells (NPCs) into iPSCs, producing inducible knock-outs of all the protein-coding genes in the mouse genome during this process. By a gRNA-abundance analysis, we were able to identify known and novel pathways that are linked to pluripotency and XCR. Using this approach, we identified the interferon gamma pathway to be involved in pluripotent stem cell reprogramming and XCR. The activation of this pathway during the early phase of reprogramming resulted in a decrease in colony number, in addition to a more efficient XCR in the pluripotent colonies. Here we will present stage-specific perturbation experiments and allele-specific RNA-Seq analysis to further delineate the mechanistic function of the interferon gamma pathway, which we demonstrate to feature in iPSC reprogramming and XCR.

Funding Source: This work was supported by the Ramón Areces Foundation and the Spanish Ministry of Science, Innovation and Universities (BFU2017-88407-P and EUR2019-103817).
Keywords: X chromosome, CRISPR screening, iPSC reprogramming

TOPIC: EARLY EMBRYO

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THE EFFECTS OF ERGOTHIONEINE ON NUCLEAR AND CYTOPLASMIC MATURATION OF PORCINE OOCYTES

Jeong, Jiyoung - *Chungbuk National University , Chungbuk, Korea*
Hyun, Sang-Hwan - *Chungbuk National University, Chungbuk, Korea*

Abstract: L-ergothioneine (EGT; 2-mercaptohistidine trimethylbetaine) is a naturally occurring amino acid and bioactive compound acknowledged as antioxidant. In this study, we investigated the effects of EGT supplementation during in vitro maturation (IVM) of porcine oocytes on nuclear maturation and cytoplasmic maturation. Each concentration (0, 10, 50, and 100 μM) of EGT was supplemented in maturation medium (TCM199-PVA) during IVM. 10 μM and 100 μM treatment groups showed a significant increase in the rate of nuclear maturation compared to the control group by examining the polar body protrusion. The polar body extrusion rates (metaphase II) were 76.2 ± 0.7 (control group), 88.0 ± 4.9 (10 μM group), 85.2 ± 3.0 (50 μM group), and 91.4 ± 2.1 (100 μM group) respectively ($p < 0.05$). After IVM, we assessed GSH and ROS levels confirming cytoplasmic maturation. GSH was significantly increased in the group treated with 50uM compared to the control group. On the other hand, it was confirmed that the ROS level decreased in the groups treated with 50uM and 100uM of EGT compared to the control group. through these results, treatment with EGT during IVM enhanced the nuclear maturation and cytoplasmic maturation of porcine oocytes. Futher studies are need-

ed to investigate the effect of EGT on the in vitro fertilization (IVF) and parthenogenetic activation (PA) of porcine oocytes.

Funding Source: This work was supported by grants from the "NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)" and "IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.
Keywords: Ergothioneine, Antioxidant, in vitro maturation

TOPIC: GERMLINE

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INTERCHROMOSOMAL INTERACTIONS OF HOMOLOGOUS STAT92E ALLELES REGULATES TRANSCRIPTION DURING STEM CELL DIFFERENTIATION

Antel, Matthew - *Cell Biology, University of Connecticut Health Center, Farmington, CT, USA*
Masoud, Madona - *Cell Biology, University of Connecticut Health Center, Farmington, CT, USA*
Raj, Romir - *Cell Biology, University of Connecticut Health Center, Farmington, CT, USA*
Li, Sheng - *Jackson Laboratory, Farmington, CT, USA*
Pan, Ziwei - *Jackson Laboratory, University of Connecticut, Farmington, CT, USA*
Mellone, Barbara - *Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA*
Inaba, Mayu - *Cell Biology, University of Connecticut Health Center, Farmington, CT, USA*

Abstract: Drosophila male germline stem cells (GSCs) constantly divide asymmetrically to produce one GSC and one differentiating gonialblast (GB). The GB enters the differentiation program in which stem cell-specific genes, such as Stat92E are quickly downregulated. GSCs are known to inherit pre-existing histones (H3 and H4), while newly synthesized histones are distributed to GBs. However, the way in which asymmetric histone inheritance contributes to the distinct fate of the two daughter cells is not known. Here we demonstrate that a change of local pairing status of homologous Stat92E loci is required for downregulation of the Stat92E gene during differentiation. Using the OligoPaint DNA fluorescent in situ hybridization (FISH) technique, we found that the interaction between homologous loci of Stat92E is always tight in GSCs and immediately loosened in GBs. When one of the Stat92E loci was absent or relocated on another chromosome, Stat92E did not pair and its expression failed to downregulate. The same defect was observed upon knock-down of global pairing factors, suggesting that pairing is likely required for switching transcriptional status. Moreover, the Stat92E enhancer element but not cis-transcription is required for the change of pairing status, indicating that the pairing change is not a consequence of transcriptional changes. Importantly, when asymmetric histone inheritance was compromised, Stat92E unpairing did not occur, suggesting that the pairing change is an intrinsically programmed process during asymmetric stem cell division. Taken together, we propose a possibility that the change of local pairing status may be a common process to rewrite gene activity status during cell differentiation.

Keywords: Germline, Stat92E, Interchromosomal

TOPIC: KIDNEY

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SINGLE CELL MULTI-MODAL METABOLOMICS IDENTIFIES METABOLIC TRAJECTORIES DURING HUMAN KIDNEY DEVELOPMENT THAT CAN ADVANCE KIDNEY ORGANOID DIFFERENTIATION

Wang, Gangqi - *Department of Internal Medicine (Nephrology), Leiden University Medical Center, Leiden, Netherlands*
Heijs, Bram - *Center of Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands*
Rietjens, Rosalie - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*
Yuan, Lushun - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*
Tiemeier, Gesa - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*
Mahfouz, Ahmed - *Leiden Computational Biology Center, Leiden University Medical Center, Leiden, Netherlands*
Giera, Martin - *Center of Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands*
Kers, Jesper - *Department of Pathology, Leiden Transplant Center, Leiden University Medical Center, Leiden, Netherlands*
Chuva de Sousa Lopes, Susana - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
van den Berg, Cathelijne - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*
van den Berg, Bernard - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*
Rabelink, Ton - *Department of Internal Medicine (Nephrology) & Einthoven Laboratory of Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Accumulating evidence demonstrates that cellular metabolism is essential in determining cell fate during development. Understanding these metabolic processes at an individual cell level during human kidney development will be crucial in optimizing strategies for kidney regeneration and repair. However, thus far it was not possible to assess dynamic metabolism assessments in an unbiased integrated way at a spatial resolution that acknowledges tissue cellular heterogeneity. We present a novel multi-omics platform to study single-cell dynamic metabolism in complex tissues by combining single-cell transcriptomics, spatial metabolomics and spatial fluxomics. We describe for the first-time cell type specific metabolic pathway trajectories using the developing human kidney as a cellular diverse and highly complex model. Each differentiation phase and cell type was identified by a unique molecular signature and metabolic profile. Single cell multi-omics data analysis allowed for the detection of unique cell states within the kidney and redefined cellular heterogeneity. For instance, decreased glycolysis and increased gluconeogenesis were observed during proximal tubule differentiation, while oxidative phosphorylation was increasingly activated during this differentiation process but derived its substrate from free fatty acids. Furthermore, we show that media supplemented with metabo-

lites identified during proximal tubular differentiation can increase differentiation of proximal tubular epithelium in hiPSC-derived kidney organoids. Our findings highlight the relevance of metabolic trajectory identification to guide stem cell differentiation in a desired direction.

Funding Source: This work is supported by Novo Nordisk Foundation grants (NNF21CC0073729) and Prof. Jaap de Graeff-Lingling Wiyadharma subsidy of the Leiden University Fund (LUF).
Keywords: Single cell metabolomics, Fetal kidney development, hiPSC-derived kidney organoids

TOPIC: NEURAL

708

SIMILARITIES AND DIFFERENCES IN ISOLATION, CRYOPRESERVATION AND LONG-TERM CULTURE BETWEEN HUMAN AND RODENT NEURAL STEM CELLS

Radoszkiewicz, Klaudia - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warszawa, Poland*
Jezierska-Woźniak, Katarzyna - *Department of Neurosurgery, University of Warmia and Mazury in Olsztyn, Poland, Olsztyn, Poland*
Mitrecic, Dinko - *Laboratory for Stem Cells, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia*
Sarnowska, Anna - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warsaw, Poland*

Abstract: Despite the fact that fetal human neural stem cells (fhNSCs) have been already used for clinical trials showing promising beneficial effect, there are several issues which still should be investigated even on preclinical studies level. Preclinical studies are often conducted with rodent NSCs in parrarel with human NSCs. Here, we present our results regarding differences in critical culture conditions such as isolation, cryopreservation and long-term culture between fhNSCs and rodent NSC. FhNSCs were obtained from fetuses after spontaneous miscarriages around 8-12 week of gestation in cooperation with the Stem Cell Research Laboratory, Department of Neurosurgery, University of Warmia and Mazury in Olsztyn, Poland according to the protocol developed jointly with our laboratory. To isolate rodent NSCs, newborn Wistar rats and C57BL/6J type mice from the Mossakowski Medicine Research Institute Animal Breeding House were used. We compared the impact of criopreservation on NSCs viability and proliferation. Subsequently, the influence of most commonly used culture medium compositions (growth factors, glutamine) on cell fate was analyzed. We confirmed our detrimental observations regarding cryopreservation protocol for fhNSCs directly after isolation on murine neural stem cells (mNSCs) and rat neural stem cells (rNSCs) culture viability and functional properties. We also pointed different impact of medium condition (growth factors, supplements) depending on the species. With this study, we would like to underlie critical points in culture that should be kept in mind even before starting experiments or interpreting the results, dedicated for further clinical settings of NSCs treatment.

Funding Source: The work was supported by the National Science Centre grant no. NCN 2018/31/B/NZ4/03172.
Keywords: neural stem cells, cryopreservation, long-term culture

TOPIC: PLURIPOTENT STEM CELLS

710

SYSTEMATIC ANALYSIS OF THE SEQUENCE-FUNCTION RELATIONSHIP UNDERLYING THE PLURIPOTENCY REPROGRAMMING ACTIVITY OF SOX17

Ho, Jessie Sik Yin - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Ho, Derek Hoi-Hang - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Jauch, Ralf - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong, Hong Kong*

Abstract: Induced pluripotent stem cells (iPSCs) are most commonly generated from somatic cells using the four Yamanaka factors OCT4, SOX2, KLF4 and c-MYC. Engineering the Yamanaka factors or their non-reprogramming family members facilitates an increase in reprogramming efficiency from fibroblasts. In particular, it has been found that engineered human SOX17 (hSOX17) variants are able to replace and even surpass wild-type SOX2 in inducing pluripotency. However, a detailed interrogation of features contributing to this activity has not yet been performed. Here we conducted deep mutational scanning to interrogate the full DNA binding domain for its capacity to convert hSOX17 into a pluripotency reprogramming factor. We demonstrate that position 57 is a crucial residue for this activity, with the substitution of the wild-type glutamic acid with nearly any other amino acid inducing pluripotency reprogramming of both mouse and human fibroblasts. Specifically, the variants incapable of contributing to reprogramming activity of hSOX17 contained proline, aspartic acid or glutamic acid at position 57. We hypothesize that position 57 may have evolved in a way that limited the activity of hSOX17 such that its native form only facilitates lineage commitment in embryonic development. We conclude that deep mutational analysis is a practical first step in re-engineering genetic factors for cell fate reprogramming. The technique could inform subsequent design of combinatorial saturation mutagenesis libraries for directed evolution in mammalian cells; as well as aid in the elucidation and understanding of the native mechanisms of transcription factors.

Keywords: reprogramming, transcription factor, protein engineering

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EFFICIENT DIFFERENTIATION OF HUMAN PRIMORDIAL GERM CELLS THROUGH GEOMETRIC CONTROL REVEALS KEY ROLE FOR NODAL SIGNALING

Jo, Kyoung H. - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Chen, Bohan - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Teague, Seth - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*

Abstract: The Human primordial germ cells (hPGCs) formation is the first step in germline specification. The PGCs are then further develop into either eggs or sperm that form around the time of implantation. This specification is a unique lineage where genetic material is passed on to the next generation and it could potential-

ly be the key developmental process in understanding totipotency. Therefore, investigating human PGC specification is critical for fundamental understanding of early human embryonic development and its practical implications in diseases. Mammalian PGC specification in mice has revealed crucial underlying knowledge. However, due to significant interspecies differences, restriction on studying post implantation embryos, and ethical reasons, in depth knowledge of human PGC specification remains elusive. Here we show that BMP4 treatment of micropatterned human pluripotent stem cells (hPSCs) induces human PGC-like cells (hPGCLCs), leading to a quantitative and reproducible in vitro model to interrogate this important developmental event. Using this model, we show that stable PGCLCs populations arise between extra-embryonic and primitive streak-like cells and they continue to mature as their protein marker expression increases for at least 96h. By interrogating signaling dynamics in hPGCLCs, we identify a previously unappreciated role for NODAL and that reduced WNT signaling is only required initially during PGCLC induction. Finally, we show that size of micropattenred hPSC colony dictates the efficiency of hPGCLC specification in a signaling dependent manner. By pharmacologically manipulating the identified signaling activity at different time points and micropatterned hPSC colony size, we dramatically improved efficiency of in vitro PGCLC induction over established state-of-the-art protocols.

Keywords: pluripotent stem cells, primordial germ cells, Signaling dynamic

POSTER SESSION I: EVEN

7:30 PM – 8:30 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: CARDIAC

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LARGE MUSCLE GRAFTS AND SUBSTANTIAL IMPROVEMENT OF HEART FUNCTION AFTER INJECTION OF HUMAN IPSC-CARDIOMYOCYTE AGGREGATES IN A NON-HUMAN PRIMATE MODEL OF MYOCARDIAL INFARCTION

Gruh, Ina - *Leibniz Research Labaratories for Biotechnology and Artificial Organs LEBAO, Hannover Medical School, Hannover, Germany*
Martens, Andreas - *Department of Cardiac, Thoracic, Transplantation and Vascular Surgery (HTTG), Hannover Medical School, Hannover, Germany*
Cebotari, Serghei - *Department of Cardiac, Thoracic, Transplantation and Vascular Surgery (HTTG), Hannover Medical School, Hannover, Germany*
Triebert, Wiebke - *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany*
Haase, Alexandra - *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany*
Schrod, Annette - *Primate Husbandry, Leibniz Institute for Primate Research, Goettingen, Germany*
Moussavi, Amir - *Functional Imaging Unit, Leibniz Institute for Primate Research, Goettingen, Germany*
Neuhaus, Vanessa - *Department of Airway Immunology, Fraunhofer ITEM, Hannover, Germany*
Veltmann, Christian - *Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany*
Jonigk, Danny - *Institute of Pathology, Hannover Medical School, Hannover, Germany*
Boretius, Susann - *Functional Imaging Unit, Leibniz Institute for Primate Research, Goettingen, Germany*
Braun, Armin - *Preclinical Pharmacology and Toxicology, Fraunhofer ITEM, Hannover, Germany*
Haverich, Axel - *Department of Cardiac, Thoracic, Transplantation and Vascular Surgery (HTTG), Hannover Medical School, Hannover, Germany*
Zweigerdt, Robert - *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany*
Martin, Ulrich - *Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany*

Abstract: Therapeutic application of hiPSC-derived cardiomyocytes (hiCMs) is considered a promising approach for heart repair. We hypothesized that the intramyocardial injection of hiCM aggregates (hiCMAs) – rather than single cells - promotes cell retention and engraftment in a cynomolgus monkey model of sub-acute myocardial infarction (MI). MI was induced by coronary artery ligation (n=11). Cardiac function was assessed via telemetric ECG recording, echocardiography and MRI. Fluorescent reporter hiCMAs of ~120 – 200 µm diameter were generated by large-scale suspension culture. Targeted differentiation yielded predominantly ventricular cardiomyocytes with >95% purity. About 5 x 107 hiCMAs were injected per heart into the peri-infarct zone 2 weeks after MI. Large hiPSC-CM grafts (>1 mm2) were identified in myocardial tissue sections both 2 weeks and 12 weeks after cell transplantation based on reporter gene expression and IF staining specific to human cardiac troponin I. Human cell grafts with prominent cross striations structurally integrated and aligned with the host tissue. In an ongoing long-term follow up of animals with reduced LVEF after MI (74% ± 5% of pre-MI function (set to 100%)), hiCMA transplantation resulted in a remarkable functional improvement of the LVEF after 12 weeks (86% ± 1%; n=3; p=0.11), in contrast to saline-injected control animals (75% ± 5%; n=2) as demonstrated by cardiac MRI. Cell graft-dependent non-life-threatening arrhythmia decreased during the three month follow-up period. In conclusion, we demonstrate the formation of substantial human heart muscle grafts 2 and 12 weeks after transplantation of relatively small cell numbers (versus comparable recent studies applying single cells), produced under largely GMP-compliant conditions. Since no teratoma formation or life-threatening arrhythmia were detected in our NHP model, the notable functional improvement argues for the timely initiation of first-in-man studies of our suggested therapeutic approach.

Funding Source: Federal Ministry of Education and Research (BMBF) to iCARE (01EK1601); German Research Foundation (DFG) to the Cluster of Excellence REBIRTH (EXC62/2) & Clinical Research Unit 311; CORTISS Foundation.
Keywords: cardiac cell therapy, preclinical animal model, bioprocess development

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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PHASE 1/2A CLINICAL TRIAL OF ALLOGENIC THRONBIM PRE-CONDITIONED WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS FOR HYPOXIC ISCHEMIC ENCEPHALOPATHY (HIE) AND INTRAVENTRICULAR HEMORRHAGE (IVH)

Joo, Kyeung Min - *Department of Anatomy and Cell Biologty, Sungkyunkwan University School of Medicine, Suwon, Korea*
Kim, Chung Kwon - *Medinno Research Institute, Medinno Inc., Seoul, Korea*
Kim, Chigu - *Division of Business Development, Medinno Inc., Seoul, Korea*

Abstract: Hypoxic ischemic encephalopathy (HIE) and interventricular hemorrhage (IVH) are major neurological disorders that could endanger the lives of neonates and result in life-long sequellae. Neuroprotective and anti-inflammatory paracrine factors of mesenchymal stem cells (MSCs) might be used to treat those diseases that have few therapeutic options. Moreover, the excretion of the paracrine factors of MSCs could be enhanced by the thrombin pre-conditioning before transplantation. The purpose of this phase 1/2a dose-escalation trial with a 3 + 3 design is to assess the safety and tolerability and to define the maximum tolerated dose of allogenic thrombin pre-conditioned Wharton's jelly-derived MSCs (WJ-MSCs) in HIE and IVH patients. Allogenic thrombin pre-conditioned WJ-MSCs (0.5 or 1.0 X 10^7 cells /1 kg, low or high dose, respectively) were transplanted into the lateral ventricle of newly-diagnosed HIE (within 168 hours after completing 72-hour hypothermia within 6 hours after diagnosis) or IVH (within 14 days after diagnosis) patients. Dose Limiting Toxicity (DLT) was defined as 1) death with unknown reason within 6 hours after injection and 2) anaphylaxis immediately after administration. In addition, safety and tolerability were monitored by serious adverse event (SAE) and tumor formation in the brain until 6 months after transplantation of MSCs. Potential efficacy was measured by neuro-development assessment such as Bayley test, radiological scale using MRI scan, and cytokines in cerebrospinal fluid and blood. Currently, three HIE patients for the low group were enrolled, and no toxicities related to allogenic thrombin pro-conditioned WJ-MSCs were observed. In the poster, the most recent results of this clinical study will be presented.

Keywords: Thrombin pre-conditioned WJ-MSC, Hypoxic ischemic encephalopathy (HIE), Intraventicular Hemorrhage (IVH)

TOPIC: EPITHELIAL_SKIN

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DEVELOPING NEW STEM CELL DIFFERENTIATION AND VALIDATION PLATFORMS FOR TRANSLATING AN INDUCED PLURIPOTENT STEM CELL THERAPY FOR THE TREATMENT OF SKIN DESEASES

Pavlova, Maryna - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Vieau, Sean - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Castillo Flores, Jocelyn - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
McGarvey, Shennea - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Balaiya, Velmurugan - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Shivananda Murthy, Meghana - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Schipper, Jessica - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
McGrath, Patrick - *Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Bush, Katie - *Medical, AVITA Valencia, CA, USA*
Hopkin, Amy - *Medical, AVITA, Valencia, CA, USA*
Bruckner, Anna - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Kogut, Igor - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Roop, Dennis - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Bilousova, Ganna - *Department of Dermatology and Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: Induced pluripotent stem cells (iPSCs) hold promise for treating skin diseases such as epidermolysis bullosa (EB). An iPSC-based gene therapy for EB involves reprogramming, gene correction, iPSC differentiation, and transplantation of genetically corrected iPSC-derived skin cells onto patients. Among challenges of this therapy are inconsistent differentiation of iPSCs into high-quality keratinocytes and unproven strategies for transplanting iPSC-derived keratinocytes (iPSC-KCs). To address the inconsistency in the quality of iPSC-KCs, we are developing an organoid-based approach for derivation of these cells. We have engineered an iPSC line with a keratin (K) 5 locus-specific knock-in of mWasabi to trace the derivation of K5+ iPSC-KCs and successfully generated skin organoids using this line. Organoid-derived K5+ iPSC-KCs are currently being characterized. For the transplantation of genetically corrected EB iPSC-KCs, strong evidence supports the use of epidermal sheets or composite skin grafts. However, the generation of epidermal sheets or composite grafts

is a lengthy and consequently expensive process. To develop a more straightforward and cost-effective skin transplantation approach, we established a novel preclinical model for grafting a human skin cell suspension onto immunocompromised mice that requires low starting cell numbers. The model involves the use of a silicone chamber and sequential transplantation of fibroblasts followed by keratinocytes. Both fibroblasts and keratinocytes are delivered in suspension in a fibrin-based gel formulation. Using this modified assay, we have successfully engrafted as low as 5 x 105 keratinocytes per 1.2 cm2 of wound area. We have also engrafted iPSC-KCs that were obtained by differentiating iPSCs as a monolayer culture. In all cases, the grafted cells formed a multilayered stratified human epidermis. We are currently investigating strategies to deliver genetically corrected EB iPSC-derived skin cells, including the cell harvesting and application techniques currently used for Spray-On-Skin™ cells developed by AVITA Medical. This approach will decrease the time to patient application vs. the time and cost it takes to grow epidermal sheets and will potentially simplify iPSC therapies for EB and other skin diseases.

Funding Source: NIH, DOD, DEBRA International, AVITA Medical
Keywords: Induced pluripotent stem cells (iPSCs), Epidermolysis bullosa (EB), Gene therapy

TOPIC: EYE AND RETINA

208

SOLUBLE CX3CL1-EXPRESSING RETINAL PIGMENTED EPITHELIUM CELLS PROTECT PHOTORECEPTORS IN A MOUSE MODEL OF INHERITED RETINAL DEGENERATION

Jong, Eric - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Hacibekiroglu, Sabiha - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Guo, Lily - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Li, Chengjin - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Ho, Margaret - *Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, ON, Canada*
Sawula, Evan - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Wallace, Valerie - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Shoichet, Molly - *Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, ON, Canada*
Nagy, Andras - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*

Abstract: Retinitis pigmentosa (RP) is an inherited retinal degenerative disease caused by mutations in over 70 genes. Because of the genetic heterogeneity, developing gene therapies for every genetic cause is unfeasible. Photoreceptor transplantation to restore vision is an alternative approach but has challenges related to cell survival and integration. Thus, we propose a combined cell and gene therapy to delay photoreceptor degeneration caused by most, if not all, inherited mutations. Human embryonic stem cells (hESC) containing our FailSafeTM system were genetically engineered to express luciferase or soluble CX3CL1 (sCX3CL1), as a prototype, via piggyBac-mediated transgenesis. We chose sCX3CL1 based on previous findings showing that exogenous sCX3CL1 can delay photoreceptor degeneration, possibly via the inhibition of microglia activation. Luciferase activity was verified by in vitro bioluminescence imaging (BLI) and sCX3CL1 expression was confirmed by RT-qPCR and western blotting. Transgenic hESC were differentiated into retinal pigmented epithelium (RPE) cells for in vivo transplantation due to their quiescent state and longevity; RPE cells were characterized by RT-qPCR, immunocytochemistry, and flow cytometry. Further, ELISA and western blotting confirmed sCX3CL1 expression and functional luciferase expression was detected in NSG mice by BLI. Luciferase- or sCX3CL1-RPE cells were injected into the subretinal space of the Rd10 mouse model of RP prior the onset of rod degeneration. We show that human RPE cells can survive in the degenerating murine retina. Moreover, immunohistochemical staining for rhodopsin revealed that eyes treated with sCX3CL1-RPE contained more rod photoreceptors in the outer nuclear layer compared to control groups, however, increased cell survival was only observed where donor cells were present. Finally, preliminary RNA expression analyses of whole retinae reveal the downregulation of pro-inflammatory genes in those treated with sCX3CL1-expressing cells. Overall, our results demonstrate the potential for a combined cell and gene therapy to prevent photoreceptor degeneration in a localized manner. This strategy could be employed to treat the cone-rich macula by using cells that produce a cone-specific trophic factor.

Funding Source: Canadian Institutes of Health Research - Foundation Grant Foundation Blindness Canada Ontario Graduate Scholarship P. K. Basu Graduate Scholar Award for Vision Health Research Vision Science Research Graduate Scholar Award
Keywords: Retinal Degeneration, Cell Therapy, Gene Therapy

TOPIC: HEMATOPOIETIC SYSTEM

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CISPLATIN-INDUCED DIFFERENTIATION OF AML CELLS: A NEW ROLE FOR AN OLD FRIEND

Alghuneim, Arwa - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Li, Yanyan - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
AlAmoodi, Asma - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
AbuElela, Ayman - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
ElShenawy, Mohamed - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
Hamdan, Samir - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
Merzaban, Jasmeen - *Bioscience, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*

Abstract: Blockage of differentiation is a characteristic feature of acute myeloid leukemia (AML) and it has been shown that the use of differentiation-inducing agents is an effective treatment strategy, especially for acute promyeloblastic leukemia (APL), a subtype of AML. All-trans retinoic acid (ATRA)'s success on this front has sparked research to find novel more broadly effective agents that can target other AML subtypes as well as deal with the heterogeneity of AML cells. Repurposing existing clinically approved drugs to find new therapeutic agents is a promising approach. In this work, we evaluate the ability of cisplatin, a chemotherapeutic drug used for solid tumors, to induce differentiation and

treat AML while specifically targeting the differentiation-resistant leukemic stem and progenitor cells. Our results show that cisplatin can induce differentiation and apoptosis in a number of AML subtypes including KG1a cells, a cell line that is used as a model of leukemic stem cells. Using in vitro assays, we illustrate that cisplatin inhibits DNA synthesis and transcription resulting in DNA adducts. Cisplatin treatment resulted in an increase in the expression of cyclin-dependent kinase inhibitor p21, a potent cell cycle regulator in normal and leukemic stem cells, which is consistent with the observed cell cycle arrest. Furthermore, cisplatin was effective in preventing the accumulation of leukemic cells in hematopoietic tissues in AML xenograft NSG mouse models. This study highlights the potential of cisplatin to be used as a therapeutic agent to treat multiple subtypes of AML through its terminal differentiation-inducing activity that is mediated by DNA damage.

Funding Source: King Abdullah university of science and technology
Keywords: Differentiation, Leukemic stem cells, Cisplatin

TOPIC: IMMUNE SYSTEM

212

OVERCOMING THE IMMUNE BARRIER IS KEY TO UNLOCKING THE POTENTIAL OF HUMAN ISLET TRANSPLANTATION FOR TYPE-I DIABETES

Schrepfer, Sonja - *Hypoimmune Platform, Sana Biotechnology Inc., San Mateo, CA, USA*
Hu, Xiaomeng - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
Gattis, Corie - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
Young, Chi - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
Olroyd, Ari - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
Chu, Elaine - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
White, Kathy - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*
Friera, Annabelle - *Hypoimmune Platform, Sana Biotechnology Inc., San Francisco, CA, USA*

Abstract: Treatment of type 1 diabetes mellitus (T1DM) via allogeneic islet transplant has limited success due to morbidities from immunosuppression (IS) and a gradual loss of engrafted islet function. We aimed to engineer human primary pancreatic islet cells (PI) that evade host immune detection and rejection without IS. Cadaveric PIs were engineered to generate two groups of islet cells. One group was engineered to knock out HLA class I and II function and overexpress CD47 (HIP) and the second to knock out HLA class I and II function (dKO). Control PI were unmanipulated and cultured identically to the other groups of cells (Wt). NSG-SGM3 mice were humanized by reconstitution with allogeneic human CD34+ cord blood; all humanized mice used in the study had >45% engraftment of human CD45+ cells. Mice were made diabetic using streptozotocin and blood glucose levels were monitored every 4 days. Firefly luciferase+ HIP, dKO, or Wt PI were transplanted into the quadriceps muscle. After 6 days, splenocytes and serum were obtained for immune analyses. HIP PI showed no measurable T cell recognition in ELISpot assays, no induction of graft-specific antibodies as assessed by flow cytometry, and were protected from NK cell and macrophage killing in vivo and in vitro. Blocking CD47 SIRPa interaction via an anti-CD47 antibody resulted in innate killing. While dKO PI (similar to HIP PI) showed no T cell activation or antibody response, they were killed by NK cells and macrophages presumably due to the induction of their “missing self” response. Wt PI showed significantly higher adaptive immune response (by ELISpot frequency) and graft-directed antibodies compared to dKO or HIP PI ($p< 0.0001$). Serial bioluminescence imaging (BLI) showed survival of all HIP PIs but rapid rejection of both dKO and Wt PI. Glucose levels (after fasting) gradually decreased after HIP PI transplantation and remained stable around 200 mg/dl after 14 days and beyond. No effect on glucose levels was seen with dKO or Wt PI transplants. To assess whether engineering alters PI function, we both assessed blood glucose levels over time and compared the survival of HIP and Wt PI via BLI in immunodeficient NSG mice. Both were similar for HIP and Wt PI. These data suggest that HIP engineering may generate HIP PI capable of persisting and functioning in diabetes patients without IS.

Keywords: Allogeneic islets, hypoimmune, immune barrier

TOPIC: MUSCULOSKELETAL

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ENHANCED MUSCLE REGENERATION FOLLOWING VOLUMETRIC MUSCLE LOSS BY A NANOCAPSULE-HYDROGEL BIOCONSTRUCT WITH STAGED RELEASE OF MULTIPLE GROWTH FACTORS

Wu, Di - *Neurology and Neurological Sciences, Stanford University, Palo Alto, CA, USA*

Abstract: Volumetric muscle loss (VML) caused by severe traumatic injuries leads to permanent loss of muscle tissue and function. Clinical interventions of VML, such as autologous muscle transfer and scar tissue debridement, are often associated with donor-site morbidity and limited tissue availability. Therefore, developing effective therapeutic strategies for VML is of great significance. Herein, we report a biomimetic bioconstruct that enables staged release of basic fibroblast growth factor (bFGF) and insulin-like growth factor-1 (IGF-1) to regulate the proliferation and differentiation of muscle stem cells (MuSCs) based on protein nanocapsule technique. Transplantation of MuSCs with this bioconstruct results in significant muscle tissue regeneration and functional recovery following VML. The histological analysis highlights de novo myofiber formation with vascularization and innervation. In addition, the muscle contraction and locomotion function assays confirm functional recovery following the bioconstruct transplantation. This work not only develops an effective therapeutic approach for VML but also provides a general platform for temporally controlling the presence of growth factors to regulate tissue regeneration.

Keywords: Volumetric muscle loss, Stem cell therapy, Stage release of growth factors

TOPIC: NEURAL

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NEURITE OUTGROWTH IS CRITICAL FOR FUNCTIONAL RECOVERY BY GRAFTS OF HUMAN IPSC-DERIVED DOPAMINE NEURONS IN A RAT MODEL

Loring, Jeanne F. - *Molecular Medicine, Scripps Research, Del Mar, CA, USA*
Hills, Rachel - *Brain Repair Group, Cardiff University, Cardiff, UK*
Mossman, Jim - *Bioinformatics, Aspen Neuroscience, Inc., San Diego, CA, USA*
Tran, Ha - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Bratt-Leal, Andres - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Williams, Roy - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Stouffer, David - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Sokolova, Irina - *Immunology and Microbiology, Scripps Research, La Jolla, CA, USA*
Sanna, Pietro - *Immunology and Microbiology, Scripps Research, La Jolla, CA, USA*
Lelos, Mariah - *Brain Repair Group, Cardiff University, Cardiff, UK*

Abstract: Transplantation of human pluripotent stem cell-derived dopaminergic (DA) neurons is an emerging therapeutic strategy for Parkinson's disease (PD). A challenge of this approach is ensuring that the transplanted cells are at an appropriate stage of development to maximize engraftment and efficacy. In anticipation of an autologous DA neuron transplant strategy, we studied human induced pluripotent stem cell (iPSC) lines derived from two people with idiopathic PD. We confirmed both cell lines' intrinsic ability to differentiate into mature DA neurons in vitro by patch-clamp electrophysiology and depolarization-induced dopamine release after long-term differentiation. To evaluate the efficacy of the cells in vivo, we transplanted cells as precursors into a hemiparkinsonian rat model and assessed recovery over 6 months by return to balanced amphetamine-induced rotations. To investigate the effect of precursor maturity on engraftment and efficacy, we differentiated both cell lines for either 18 (d18) or 25 days (d25) before transplantation. We found comparable survival of grafts with similar DA neuron content from both cell lines at both stages of maturity. However, behavioral analysis revealed that for both cell lines only one of the stages of maturation, d18, resulted in recovery of motor impairments. Histological analysis revealed that there were striking and consistent differences in graft neurite outgrowth between grafts from the two stages of maturation. The functional grafts, from d18 cells, had more outgrowth than the non-functional grafts from d25 cells. These results show that graft-host integration, and not DA neuron content, is required for functional recovery. A time course of gene expression during in vitro differentiation revealed differences in genes associated with neuronal development and neurite plasticity, offering insights into the optimal developmental stage to maximize graft efficacy from each patient-specific iPSC line for autologous therapy.

Funding Source: Parkinson's UK (F-1502), Summit for Stem Cell Foundation, California Institute for Regenerative Medicine (CIRM; CL1-00502, RT3-07655, GC1R-06673-A, DISC2-09073, DISC2P-11595). NIH (DA046170, DA046204-04, DA043268).
Keywords: Parkinson disease, Neuron replacement therapy, Autologous iPSCs

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DEVELOPMENT AND EFFICACY OF IPSC-DERIVED NEURAL PROGENITOR CELLS GENETICALLY ENGINEERED TO PRODUCE GDNF AS A THERAPY FOR ALS

Wu, Amanda K. - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Laperle, Alex - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Moser, Vanessa - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Peykar, Eden - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Lawless, George - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Avalos, Pablo - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Svendsen, Clive - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting approximately 30,000 individuals in the US at a given time. ALS is characterized by progressive spinal and cortical motor neuron loss, with paralysis and death typically within 4 years of diagnosis. There is no cure, and FDA-approved drugs only minimally slow progression. Transplanting supportive glial cells engineered to release glial cell line-derived neurotrophic factor (GDNF) is a promising treatment. Our group has generated and extensively characterized human fetal-derived neural progenitor cells (NPCs) lentivirally transduced to stably produce GDNF. These cells engraft in the spinal cord and slow the loss of choline acetyltransferase (ChAT)-positive motor neurons in the SOD1G93A rat model of ALS. We recently completed a Phase 1/2a trial that showed the safety of delivering these cells to the spinal cords of ALS patients as a first-in-human cell and gene therapy approach. While promising, the use of these GDNF-releasing NPCs is constrained by ethical concerns with fetal tissue and the availability of starting material. Here, we assess human-induced pluripotent stem cells (iPSC) as an alternative, renewable cell source that can differentiate into NPCs. We have generated iPSC-derived NPC lines transduced to express GDNF (iNPC-GDNF). Single-cell RNA-seq showed that iPSC- and fetal-derived NPCs expressed neural progenitor markers, however, the majority clustered separately. To establish the functional effect of iNPC-GDNF, we unilaterally transplanted cells in the SOD1G93A rat lumbar spinal cord, with euthanasia at disease onset. While transplants did not alter disease onset, hindlimb motor function was significantly improved in the treated limb compared to non-transplanted side and non-treated controls. Immunohistochemistry (IHC) showed engraftment and GDNF production. The number and size of ChAT+ motor neurons (>400 um2) were both significantly increased in the grafted region of treated rats (n=10) compared to non-transplanted side and non-treated controls (n=7), suggesting cells provide a neuroprotective effect. In order to evaluate long-term safety and tolerability, iNPCs transplanted into athymic nude rats for 9 months are being analyzed by IHC for survival, proliferation, and cellular phenotype.

Funding Source: Board of Governors Regenerative Medicine Institute
Keywords: iPSC, GDNF, SOD1

TOPIC: PLURIPOTENT STEM CELLS

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EXPANSION AND DOWNSTREAM DIFFERENTIATION POTENTIAL OF HUMAN PLURIPOTENT STEM CELLS (HPSCS) MAINTAINED IN GMP ANIMAL ORIGIN-FREE MEDIA

Snyder, Kimberly A. - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Hoang, Thuy - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Neef, Olivia - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
LeBlanc, Noëmie - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Gill, Jaspreet - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Hunter, Arwen - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: Successful transition of hPSC-based therapies to the clinic is reliant on demonstrating safety of the cell product and of the components in contact with the product throughout the manufacturing process. To ease this transition, TeSR™-AOF, a robust hPSC expansion medium, is manufactured under cGMP and was developed with animal-free raw materials with traceability to the secondary level of manufacturing. TeSR™-AOF was optimized to improve plating efficiency and expansion of hPSCs compared to low-protein formulations. Typically, plating efficiency in TeSR™-AOF was enhanced by 27.1% ± 4.71% (mean ± STDEV; n=3 cell lines); however, in select hPSC lines with historically low plating efficiency in low-protein media formulations, the plating efficiency was improved by 80 to 140% (n=2 cell lines) in TeSR™-AOF. To assess the regenerative potential and differentiation efficiency of hPSCs maintained in TeSR™-AOF, we assessed compatibility with several clinically-relevant directed differentiation protocols. Efficient differentiation of hPSCs maintained in TeSR™-AOF to cardiomyocytes was demonstrated using the STEMdiff™ Cardiomyocyte Differentiation Kit, with 87.7% ± 5.48% of cells staining positive for cardiac troponin T by flow cytometry (n=2 cell lines). hPSCs in TeSR™-AOF were also shown to efficiently differentiate to megakaryocytes using the STEMdiff™ Megakaryocyte Differentiation Kit, with 79.2% ± 5.76% CD41+/CD42+ double-positive cells (n=2 cell lines). hPSCs in TeSR™-AOF were shown to differentiate to 99.9% ± 0.025% TuJ+, 64.9% ± 4.23% TH+ dopaminergic neuron cells using the STEMdiff™ Midbrain Neuron System (n=1 cell line). Compatibility of hPSCs expanded in TeSR™-AOF with gene editing was confirmed using the ArciTect™ CRISPR-Cas9 genome editing system. GFP-tagged hPSCs cultured in TeSR™-AOF demonstrated 68.6 ± 2.03% GFP knock-out efficiency and 9.17 ± 1.77% ssODN GFP to BFP knock-in efficiency (n=2 cell lines). hPSCs in TeSR™-AOF had a cloning efficiency of 33.7 ± 8.04% (n=3) when supplemented with a cloning enhancing supplement, CloneR™2. In summary, TeSR™-AOF was designed with quality and safety in mind and formulated to improve attachment efficiency, consistency and reproducibility, whilst enabling versatile workflows and preserving high quality hPSCs in long term culture.

Keywords: cGMP, Differentiation, Pluripotent

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

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ASSESSMENT OF QUALITY OF MESENCHYMAL STEM CELLS DERIVED FROM ADIPOSE TISSUE AND PREPARED FOR CLINICAL APPLICATIONS ACCORDING TO VALIDATED PROTOCOLS

Sypecka, Monika - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Center, Warszawa, Poland*
Chodkowska, Magdalena - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warszawa, Poland*
Krześniak, Natalia - *Centre of Postgraduate Medical Education, Prof. W. Orlowski Memorial Hospital, Department of Plastic and Reconstructive Surgery, Warsaw, Poland*
Sarnowska, Anna - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warsaw, Poland*

Abstract: In recent years, the number of active clinical trials concerning mesenchymal stem/stromal cells (MSCs) has significantly increased. This may be due to the fact that MSCs exhibit many therapeutical properties – these cells, which are obtained from relatively accessible sources (for example adipose tissue, umbilical cord, bone marrow), have been shown to manifest immunosuppressive and neuroprotective properties. It is because of these features that MSCs have already been used in clinical trials regarding neurological disorders like amyotrophic lateral sclerosis (ALS) or Parkinson's disease. In our study, we chose to examine the quality of obtained cells and isolation efficiency. We managed to describe specific conditions of cell cryopreservation, preparation and transportation, which in our opinion are required for obtaining a medicinal product of highest quality destined for clinical use. We closely analysed cell viability under different conditions, including the use of different transport mediums and different duration of transportation of the medicinal product to the clinic. Adipose derived mesenchymal stem/stromal cells (ASCs) obtained by our team showed features characteristic for MSCs – they exhibited a fibroblast-like morphology in vitro, expressed CD73, CD90, CD105 markers and underwent mesodermal differentiation. They also have the ability to form colonies. In MSCs clinical trials, the site of cell injection is an important element. As different injection sites cause the cells to enter different environments, this may influence the phenotype of the cells. Therefore, we decided to investigate the influence of cerebrospinal fluid (CSF) obtained from patients on ASCs. After being exposed to CSF for 7 days, the cells expressed markers characteristic for neural cells and neural stem cell line: NG2, A2B5, nestin, β-III-tubulin, S-100-β. We also observed a slight change in morphology when it comes to ASCs cultured in the presence of CSF – the cells became elongated and formed axon-like protrusions. It seems that, after being exposed to CSF, ASCs undergo neural differentiation, which is a crucial information considering the application of MSCs therapy in neurodegenerative diseases. Our results show that MSCs exhibit great therapeutic potential when it comes to treating neurological disorders.

Funding Source: 2020/ABM/01/00014-00
Keywords: mesenchymal stem/stromal cells, cerebrospinal fluid, neural differentiation

TOPIC: EYE AND RETINA

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BIOMIMETIC ELECTRICAL STIMULATION AS A NOVEL TECHNOLOGY TO ENHANCE PLURIPOTENT STEM CELL-DERIVED RETINAL ORGANOID DEVELOPMENT, MATURATION AND FUNCTIONALITY

O'Hara-Wright, Michelle - *Stem Cell Medicine Group, Children's Medical Research Institute/University of Sydney, Milsons Point, Australia*
Smith, Grady - *Stem Cell Medicine Group, Children's Medical Research Institute, Sydney, Australia*
Lim, Benjamin - *Stem Cell Medicine Group, Children's Medical Research Institute, Sydney, Australia*
Wong, Emilie - *Stem Cell Medicine Group, Children's Medical Research Institute, Sydney, Australia*
Aryamanesh, Nader - *Embryology, Children's Medical Research Institute/University of Sydney, Australia*
Gonzalez-Cordero, Anai - *Stem Cell Medicine Group, Children's Medical Research Institute/University of Sydney, Australia*

Abstract: Pluripotent stem cell-derived retinal organoids provide ideal systems for modelling retinal disease, screening therapies and generation of cells for transplantation. However, a lack of mature photoreceptor cells and robust demonstration of functionality prevents true imitation of the in vivo retina. Current culture conditions fail to account for all physiological cues exhibited in vivo, such as exogenous electrical fields generated from separation of ions. Intrinsic bioelectricity is implicated in key processes such as differentiation, migration and regeneration. Therefore, we hypothesise that introduction of biomimetic electrical fields in vitro will enhance retinal organoid development and functionality. We aim to test this via application of electrical stimulation using an in-house bio-stimulation chamber. Various biomimetic stimulation parameters were applied to organoids at key stages of development. Multi-omics analysis of stimulated organoids uncovered molecular candidates involved in bioelectric-driven processes. Mass spectrometry demonstrated modulation of extracellular matrix proteins. Stage-dependent effects on cell composition, proliferation and differentiation were revealed in transcriptomes of organoids stimulated during early, mid, or late periods of development. Flow cytometry and immunohistochemistry confirmed this, finding a significant 24.2% increase in photoreceptor yield in organoids treated with optimal conditions during mid-stage development. Importantly, no significant increase in toxicity was observed. We provide first demonstration of electrical stimulation to retinal organoids, suggesting electrical field modulation may accelerate differentiation potential and thereby functionality. As a novel tool, application of exogenous electrical fields to organoid systems should promote more accurate physiological niches. This offers more robust stem-cell derived models, increasing opportunities for cell, gene and pharmacological therapies.

Funding Source: Luminesce Alliance
Keywords: Retinal, Organoid, Electrical Stimulation

TOPIC: KIDNEY

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TOWARDS A PERSONALIZED KIDNEY-ON-A-CHIP DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

Jaeschke, Michelle - *Research and Development, TissUse GmbH, Berlin, Germany*
Faust, Daniel - *Research and Development, TissUse GmbH, Berlin, Germany*
Koenig, Leopold - *Research and Development, TissUse GmbH, Berlin, Germany*
Nguyen, Nhutuyen - *Research and Development, TissUse GmbH, Berlin, Germany*
Ramme, Anja - *Research and Development, TissUse GmbH, Berlin, Germany*
Marx, Uwe - *Research and Development, TissUse GmbH, Berlin, Germany*
Dehne, Eva-Maria - *Research and Development, TissUse GmbH, Berlin, Germany*

Abstract: Our understanding of the mechanisms involved in the development and progression of renal disease is still limited due to the lack of functional in vitro models that can accurately emulate human physiological processes. Microphysiological systems enable the long-term co-cultivation of multiple cell types under nearly physiological conditions and can thereby bridge the gap between conventional in vitro models and human patients. These microphysiological systems are, however, typically equipped with heterogenous cell populations sourced from multiple donors with diverse genetic backgrounds, which limits their applications in personalized medicine and patient-specific disease modeling. In this study, we generate a personalized and autologous kidney-on-a-chip that encompasses human induced pluripotent stem (iPS) cell-derived podocytes and proximal tubule epithelial cells from a single donor. The renal cells are seeded into the HUMIMIC Chip4, which enables the long-term co-cultivation of the renal model with up to three additional organ equivalents with a defined fluid flow and shear stress. The final maturation of the iPS cell-derived podocytes and tubular cells occurs within the multi-organ-chip, thereby allowing the cells to form a barrier that enables glomerular filtration and tubular reabsorption. After the renal cells' final maturation, the co-culture with autologous equivalents of the small intestine, liver and brain can be maintained for at least 14 days. The kidney-on-a-chip exhibits a stable metabolism, a cellular barrier that prevents albumin from entering the excretory circuit, and the cells demonstrate a steady expression of key podocyte and tubular markers. When the employed organ models are differentiated from iPS cells derived from a single patient, the HUMIMIC Chip4 represents a personalized multi-organ-chip. This patient-on-a-chip can be used for elaborate efficacy studies and the development of personalized therapies. Therefore, the developed autologous kidney-on-a-chip could not only advance future studies of renal disease mechanisms, but it could also pave the way towards personalized medicine.

Keywords: microphysiological system, iPS cell-derived kidney-on-a-chip, multi-organ-chip

TOPIC: NEURAL

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THE FATE OF IPSCS-DERIVED DOPAMINERGIC NEURON PRECURSORS AFTER TRANSPLANTED INTO THE STRIATUM OF RATS WITH 6-OHDA-INDUCED PARKINSONISM

Lebedeva, Olga S. - *Cell Biology Laboratory, FRCC PCM, Moscow, Russia*
Voronkov, Dmitry - *Brain Research Department, Research Center of Neurology RAS, Moscow, Russia*
Lebedeva, Olga - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Stavrovskaya, Alla - *Brain Research Department, Research Center of Neurology RAS, Moscow, Russia*
Bogomiakova, Margarita - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Olshanskiy, A - *Brain Research Department, Research Center of Neurology RAS, Moscow, Russia*
Kopylova, Irina - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Gushchina, Anastasia - *Brain Research Department, Research Center of Neurology RAS, Moscow, Russia*
Simonova, Anastasia - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Ruchko, Evgeny - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Illarioshkin, Sergey - *Research Center of Neurology RAS, Moscow, Russia*
Eremeev, Artem - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*
Lagarkova, Marya - *Cell Biology Laboratory, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia*

Abstract: In patients with Parkinson's disease, 50% to 90% of dopaminergic neurons in the substantia nigra may be lost. In this regard, cell replacement therapy may become a promising treatment method. The induced pluripotent stem cells (iPSCs) capable of differentiation into all types of somatic cells can serve as a cell source. However, there is scarce information on graft's cellular composition formed during in vivo differentiation of transplanted iPSC-derived neuronal precursors. In the present study, we differentiated iPSCs into precursors of midbrain dopaminergic neurons using a previously developed protocol. After immunocytochemical characterization, the progenitors were transplanted into the striatum of rats with 6-OHDA-induced parkinsonism. In parallel, neuronal progenitors of dopaminergic neurons differentiated from isogenic iPSCs with beta-2-microglobulin knockout were transplanted to another group of model animals. The derivatives of such iPSCs are low immunogenic since they do not have HLA class I molecules on their surface. Although such cell products do not entirely avoid the immune response, they significantly reduce it, allowing the use of milder immunosuppressive therapy. We monitored the in vivo differentiation for six months and compared it to standard in vitro differentiation. The dynamics of neuron maturation in the graft were assessed. Also, the host systemic inflammatory response and the biodistribution of injected cells were studied. The efficiency of in vivo differentiation in TH+ neurons was 3-7%. Surprisingly, we found that a significant part of the progenitors was differentiated into glial cells. We observed host striatum neurons growing into the transplant area as early as 3 months after injection. Thus, we approached the preclinical testing of the cell products, having characterized in detail the dynamics of maturation and the composition of the graft and the immune response for both wild-type iPSCs derivatives and derivatives lacking HLA class I expression.

Funding Source: This work was supported by grant 075-15-2019-1669 from the Ministry of Science and Higher Education of the Russian Federation.
Keywords: cell therapy, Parkinson's disease, Dopaminergic neurons progenitors

TOPIC: PANCREAS

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TOWARDS A GMP-COMPLIANT PROTOCOL FOR THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO BETA CELLS FOR THE TREATMENT OF TYPE 1 DIABETES

Rajaei, Bahareh - *Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*
Carlotti, Françoise - *Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*
de Koning, Eelco - *Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*
Muñoz García, Amadeo - *Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*

Abstract: The generation of insulin-producing cells from human pluripotent stem cells (hPSC) in vitro provides an unlimited cell source for drug discovery and cell replacement therapy for diabetes. Here we report the generation of insulin positive cells under GMP (Good Manufacturing Practice)-compliant conditions. Experiments were performed on both research-grade and GMP-grade human embryonic stem cell lines. We adapted a seven-stage (30-day) differentiation protocol to generate hPSC-derived beta islet cells in a 3D suspension culture system. Differentiation efficiency was monitored by flowcytometry. Stage 7 cells were evaluated in vitro by single cell RNA sequencing and glucose-stimulated insulin secretion and in vivo by intraperitoneal glucose tolerance test after transplantation under the kidney capsule in immunodeficient mice. Human C-peptide was determined by ELISA. Cells acquired a pancreatic progenitor phenotype at stage 4 (day 12), characterized by the co-expression of PDX1/NKX6.1 (40-50%). At the end of stage 7 (day 30), C-peptide-positive beta cells (30-45%) and glucagon-positive alpha cells (5-20%) were present. Following transplantation of stage-7 clusters into normoglycemic mice, stimulated human C-peptide secretion level increased over time and reached 168± 93 pmol/L (n=16) and 661± 261 pmol/L (n=8) at day 60 and 90 respectively, indicating further maturation of the cells in vivo. Single-cell transcriptomics data allowed us to identify and characterize all cell types present in the stage 7 clusters. We can generate glucose-responsive insulin secreting beta cells using research-grade and GMP-grade cell lines. These cells further mature after transplantation in vivo.

Keywords: human pluripotent stem cells (hPSC), C-peptide-positive beta cells, GMP

POSTER SESSION I: EVEN

7:30 PM – 8:30 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

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UTILISING FUNCTIONAL HYDROGELS TO MODEL EMBRYONIC HEART DEVELOPMENT IN VITRO

Scaglioni, Dominic - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*
Magnussen, Michael - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*
Di Biagio, Daniele - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*
Giomo, Monica - *Department of Industrial Engineering, University of Padova, Italy*
Moulding, Dale - *Light Microscopy Core Facility, UCL Great Ormond Street Institute of Child Health, London, UK*
Luni, Camilla - *Department of Civil Engineering, Environment and Materials, University of Bologna, Italy*
Pavan, Piero - *Department of Industrial Engineering, University of Padova, Italy*
Urciuolo, Anna - *Department of Women's and Children's Health, University of Padova, Italy*
Eaton, Simon - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*
De Coppi, Paolo - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*
Elvassore, Nicola - *Department of Stem Cells and Regenerative Medicine, UCL Great Ormond Street Institute of Child Health, London, UK*

Abstract: During development, the heart transforms from a linear tube to a multichambered organ. This process requires orchestration of both mechanical and chemical cues between primordial cardiac tissues and the surrounding environment. Errors in these early stages of human cardiogenesis are known to cause congenital heart defects, however, existing in vitro models are insufficient to address the morphometric abnormalities that occur in vivo. Utilising a recently developed bioprinting technology that enables the photo-crosslinking of biopolymers within hydrogels, we have created a novel 3D tissue-engineered in vitro model of the primordial heart tube. We are able to finely tune the stiffness of the hydrogel over biologically matched ranges (1kPa-20kPa) by modulating both the laser power and the number of printing cycles. This enables patterning of mechanical properties with micrometric resolution allowing us to generate regional domains with specific elastic modules. The bioprinted scaffold enables robust cardiomyocyte differentiation from human iPSCs with the formation of a single cell layer around the hydrogel scaffold with correct polarisation and organisation with representative morphology and geometry to what is observed in vivo. The mechanical properties of the tubes can be designed to be compliant with cardiomyocyte contraction with corresponding changes in the luminal cross-section depending on scaffold stiffness. We now envision the creation of controlled small molecule concentration gradients across the different axis of the linear heart tube in an attempt to recreate the patterning process that occurs in vivo. We are using this technology to investigate the process of linear heart tube looping, developmental asymmetry and trabeculation. Ultimately, an in vitro model of early-stage human heart development will provide a powerful testbed to explore the mechanism of cardiogenesis and the possibility to develop novel therapies for congenital heart malformations.

Funding Source: This work primarily funded by the British Heart Foundation
Keywords: cardiogenesis, bioengineering, developmental biology

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STRUCTURAL ORGANIZATION AND FUNCTIONAL ANALYSIS OF COMPOUND RESPONSES IN 3D HUMAN IPSC-DERIVED CARDIAC TRI-CULTURE MICROTISSUES

Tong, Zhisong - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*
Crittenden, Caroline - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*
Lim, Angeline - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*
Himmerich, Sarah - *Innovation and New Technology, FUJIFILM Cellular Dynamics, Inc., Middleton Town, WI, USA*
Rieger, Cara - *Research and Development, FUJIFILM Cellular Dynamics, Inc., Middleton Town, WI, USA*
Vaidyanathan, Ravi - *Innovation and New Technology, FUJIFILM Cellular Dynamics, Inc., Middleton Town, WI, USA*
Carlson, Coby - *Innovation and New Technology, FUJIFILM Cellular Dynamics, Inc., Middleton Town, WI, USA*
Sirenko, Oksana - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*

Abstract: The human heart is a complex organ providing highly regulated processes of moving blood through the body. The adult human ventricle is comprised of cardiomyocytes, endothelial cells, fibroblasts and other cell types. Though cardiomyocytes make up 75% of the total volume of human ventricle, it only constitutes 50% of the total cell number. Recent publications show that tri-cellular co-culture microtissues of cardiomyocytes, endothelial cells and cardiac fibroblasts all derived from human iPSC enhance the maturation and functional activity of cells compared to 2D cardiomyocytes and thus more closely mimics actual heart physiology. In the study, we used a tri-culture model created by mixing iPSC-derived cardiac cells with primary adult fibroblasts and iPSC-derived endothelial cells at 70:20:10 ratio in ultra-low attachment (ULA) plates. We used a Biomek liquid handling system for cell plating and subsequent media exchange (every 2 days). 3D microtissues were formed within 48 hours and started to contract spontaneously and regularly on Day 5. We investigated the functional activity of microtissues by recording calcium oscillations after addition of calcium dye using a fast kinetic fluorescence recording instrument (FLIPR Penta). We tested the response of the microtissues to a number of known modulators of cardiac activity. Importantly, we found that molecules like isoproterenol significantly accelerated the oscillation rate and also increased the peak amplitude. Several other compounds, including hERG inhibitors, ion channel blockers, or beta-blockers,

demonstrated changes in the Ca2+oscillation patterns consistent with expected mode of action. Additionally, we used high content imaging to characterize the structure and morphology of our 3D microtissues with an IXM-HT.ai automated confocal imaging system. Different cell types were immunostained using anti-Troponin T (for cardiomyocytes), VE-Cadherin (for endothelial cells), and COL1A1 (for fibroblasts) antibodies. The 3D structure of microtissues were reconstructed and analyzed using MetaXpress image analysis software. The data presented here highlight the utility and biological relevance of using iPSC-derived cell types in 3D microtissues as promising model for measuring compound effects on human cardiac tissues in high throughput format.

Keywords: High-Content Imaging, FLIPR, Cardiac

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N-TERMINALLY TRUNCATED HERG CHANNELS GENERATED BY KCNH2 FRAMESHIFT MUTATION (C.453DELC) INDUCES LQT PHENOTYPE IN PATIENT-DERIVED IPSC-CMS

Park, Na Kyeong - *Physiology, Seoul National University, Seoul, Korea*
Kim, Sung Joon - *Physiology, Seoul National University, Seoul, Korea*
Choi, Seong Woo - *Physiology, Dongguk University, Gyeongju, Korea*

Abstract: Patient-specific cardiomyocytes from human induced pluripotent stem cells (hiPSC-CMs) are valuable for studies in the inherited cardiac diseases. A recent study reported single nucleotide C deletion mutation in the exon 3 of KCNH2 gene (c.453delC-KCNH2, p.151Pfs +15X in hERG) associated with LQT syndrome (Park JK et al., 2013). Since the 453delC-KCNH2 resulted the frameshift of the coding sequences, a premature termination of translation at the N-terminal region was suggested. However, there is an additional initiation codon next to the mutated residue. To elucidate the precise mechanism of LQT phenotype, we performed whole-cell patch clamp and immunoblot assay in 453delC-KCNH2 hiPSC-CMs and HEK293 cells transfected with 453delC-KCNH2. The 453delC-KCNH2 hiPSC-CMs showed significantly prolonged action potential duration (APD) and reduced density of the rapidly activating delayed rectifier K+ current (IKr). The density of IhERG in HEK293 cells transfected with 453delC-KCNH2 was 10 % of the wild type (WT) IhERG. However, voltage dependence of activation, voltage dependence of inactivation, and deactivation kinetics of 453delC-KCNH2 were not significantly different from those of WT. To study the interaction between WT and mutant, the equimolar amounts of WT and 453delC cDNA were transfected into HEK293 cells. The current density of WT/453delC channels was half of that from the WT channel alone, indicating insignificant dominant negative effect. Immunoblot analysis of WT channel showed 150 kDa of core-glycosylated form and 180 kDa of fully-glycosylated channel. Interestingly, 453delC-KCNH2 overexpressed cells showed 135 kDa and 160kDa suggesting that the translation of shorter form, i.e. N-terminal truncated hERG, actually occurred with subsequent glycosylation. Nevertheless, the markedly reduced IhERG and the prolonged APD indicated functionally impaired state of 453delC-KCNH2, consistent with the LQT2 phenotype.

Keywords: Long QT syndrome, KCNH2, Patient-derived iPSC

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KCNQ1 RESIDUAL PATERNAL-ALLELE IMPRINTING IN HIPSC-DERIVED CARDIOMYOCYTES IS LOST UPON MATURATION IN TRI-CELLULAR CARDIAC MICROTISSUES REVEALING MUTATION EFFECTS

Campostrini, Giulia - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Ward-van Oostwaard, Dorien - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Verkerk, Arie - *Heart Centre, Department of Clinical and Experimental Cardiology, Academic University Medical Center, location AMC, University of Amsterdam, Amsterdam, Netherlands*
Mummery, Christine - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Bellin, Milena - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: KCNQ1 gene encodes for the cardiac potassium channel mediating the slow delayed rectifier current IKs and its mutations are associated with long-QT syndrome type 1 (LQT1), a cardiac arrhythmia often leading to sudden cardiac death. KCNQ1 expression is regulated by genomic imprinting: it is maternal allele-specific in all tissues, but becomes bi-allelic in the heart during development. Human iPSC-derived cardiomyocytes (hiPSC-CMs) have been widely used to model LQT1; however, residual KCNQ1 imprinting in hiPSC-CMs due to epigenetic memory and their immature state may hamper proper evaluation effects of mutations in the paternal allele. Here, we analyzed different hiPSC-CM lines and showed by ddPCR that all presented unbalanced KCNQ1 allelic expression. We then used hiPSC-CMs from a LQT1 patient carrying a KCNQ1 mutation on the paternal allele and compared their functional properties with the isogenic corrected line. Patch clamp analysis showed no difference in the action potential duration (APD) between the two lines, confirming that imprinting was masking the mutation effect. We then matured hiPSC-CMs in cardiac microtissues (MTs) with hiPSC-derived cardiac fibroblasts and endothelial cells. The tri-cellular spheroid environment was previously shown to promote maturation and induce upregulation of KCNQ1. Here, we observed an increased expression of the paternal allele compared to 2D-cultured hiPSC-CMs. Besides, we found a reduction in the expression of kcnq1ot1, the long non-coding RNA inducing paternal allele repression. LQT1 hiPSC-CMs dissociated from MTs showed prolonged APD compared to the corrected line, thus revealing the mutation effect. Importantly, the increase in KCNQ1 expression allowed to measure the slow component of IKs and demonstrate that longer APD was caused by IKs reduction in LQT1 compared to corrected hiPSC-CMs. Our findings show residual KCNQ1 imprinting in immature hiPSC-CMs, potentially leading to underestimate functional effects of pathological variants carried by the paternal allele. This is overcome by maturation of hiPSC-CMs in tri-cellular cardiac microtissue which promotes KCNQ1 bi-allelic expression. This study brings attention to the epigenetic regulation of hiPSC models and demonstrates the utility of using matured hiPSC-CMs as in vitro preclinical model.

Funding Source: European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie (838985) and European Research Council (101001746); Netherlands Organisation for Health Research and Development ZonMW (114022504)
Keywords: cardiac microtissues, KCNQ1, imprinting

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FAT-INDUCED ELECTROPHYSIOLOGICAL ALTERATIONS ON HUMAN IPSC-DERVICED CARDIOMYOCYTES IN CARDIOMYOPATHY MODELING WITH ADIPOCYTES

Morrissette-McAlmon, Justin - *Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*
Boheler, Kenneth - *Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*
Teuben, Roald - *Cardiology, Johns Hopkins University, Baltimore, MD, USA*
Tung, Leslie - *Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*
Xu, William - *Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*

Abstract: The native myocardium is surrounded by epicardial fat that has been postulated to serve as a cardioprotective layer. Conversely, in certain cardiac diseases like arrhythmogenic cardiomyopathy (ACM) infiltration of fat into the myocardium can promote cardiac injury and lead to heart dysfunction, arrhythmias, and heart failure. To model this situation, we developed a co-culture model of adipocytes and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) originating from both healthy and clinically diagnosed ACM individuals. An ACM mutant line for plakophilin-2 (PKP2) and another for desmoglein-2 (DSG2) [the two most common causal variants] were compared against two wild-type hiPSC-CM lines. Differences in electrophysiology (voltage, calcium and ion channel gene expression), expression profiles of desmosomal genes PKP2, DSG2 and JUP, and presence of lipids amongst these lines were measured for both direct co-culture and conditioned media. Compared to the wild-type lines, PKP2 mutant cells had prolonged action potential duration (APD), reduced conduction velocity (CV), and increased SCN5A expression. However, calcium transients were relatively unaltered. With the addition of adipocytes, APD increased, CV and calcium transients remained the same, RNA expression of PKP2 increased, and DSG2, JUP, and SCN5A (fast sodium channel) expression decreased. Compared with the wild-type lines, DSG2 mutant cells differed in a manner akin to the PKP2 mutant cells in terms of prolonged APD and expression of desmosomal and SCN5A genes, although CV did not change. Upon co-culture with adipocytes, APD, SCN5A expression and DSG2 expression increased, but no changes were seen in CV, calcium transients or PKP2 and JUP expression. For both mutant cell lines, the effects of conditioned media were largely similar to those obtained with direct co-culture. In summary, significant differences were observed between ACM and wild-type hiPSC-CMs in terms of their electrophysiology and expression profiles of desmosomal genes. The main effect of addition of adipocytes was prolongation of APD in both mutant lines, as well as increased expression of the mutant desmosomal gene. Our findings suggest that paracrine signaling may be mainly responsible for adipocyte effects on myocyte electrophysiology.

Funding Source: NIH R01 HL120959, HL152249
Keywords: ARVC/ACM, iPSC-CMs, In-Vitro Modeling

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EVALUATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN CARDIAC SAFETY ASSAYS

Yeon, Seunghee - *Seoul, Korea*
Kim, Su-Bin - *Department of Commercializing Stem Cell Technology, Nexel, Seoul, Korea*
Lee, Seung-Yeon - *Department of Commercializing Stem Cell Technology, Nexel, Seoul, Korea*
Yoon, Seulgi - *Department of Commercializing Stem Cell Technology, Nexel, Seoul, Korea*

Abstract: Cardiac toxicity is a major cause of compound attrition during drug development as well as for the withdrawal of drugs already on the market. Although current safety guidelines have successfully eliminated hERG-related risk issues there are many concerns over the accuracy of the assay as hERG-block does not always equate to QT prolongation in patients. With recent advances in the stem cell field, it is now possible to generate human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) that recapitulate the native behavior and accurately assess the proarrhythmic potentials of candidate drugs. At present, these cells are being actively investigated with high-throughput technology, especially through the ICH guideline revision (CiPA initiative), for their potential use as novel assays for preclinical toxicity testing. In this study, we used Cardiosight®-S hiPSC-CM could be used for in vitro drug screening. Upon exposure to CiPA Phase II Validation Study Compounds identified as High, Medium, or Low risk for manifesting human TdP, the cells showed an expected ability to predict cardiotoxic effects. Expected prolongations or shortenings of the field potential duration or modified beating behaviors illustrate that the expected drug responses were reproduced with Cardiosight®-S hiPSC-CM, suggesting high physiological relevance of the cells. Furthermore, we optimized 96 well conditions of not only field potential but also impedance (contraction) suitable for toxicity screening in vitro. We concluded that toxicity test using Cardiosight®-S hiPSC-CM could be a good alternative source for determining cardiac risk earlier in drug discovery and preclinical study to assess compounds' cardiac liability.

Keywords: CiPA initiative, cardiotoxicity, drug screening

TOPIC: EARLY EMBRYO

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TOTAL RNA ANALYSIS OF MOUSE GASTRULOIDS USING VASA SEQUENCING AND THEIR COMPARISON TO MOUSE EMBRYO

Dommann, Noëlle A. - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Yildiz, Mehmet - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Martinez, Clara - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Dijkers, Pascale - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Geijsen, Niels - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Alemany, Anna - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Gastruloids are aggregates of embryonic stem cells that display an embryo-like organization and allow to closely recapitulate the first steps of the mammalian development in vitro. Gastruloids are an ideal model system for the early post implantation development as they can be grown in large quantities and display key features of axial organization and germ-layer specification, as well as closely mimic somitogenesis. However, the role of non-coding transcript biotypes such as snoRNA, piRNA or long non-coding RNA remains unknown in gastruloids. To investigate this with single-cell resolution, here we use our recently established total RNA-sequencing technology called Vast transcriptome Analysis of Single cells by dA-tailing (VASA-seq) which provides a wider spectrum of RNA biotypes as compared to standard single cell RNA sequencing. Vasa-seq RNA biotypes include long non-coding, short non-coding and non-polyadenylated transcripts, therefore allowing the identification of of novel transcriptomic signatures in the different embryonic cell types and looking at different time points of gastruloids. Moreover, VASA-seq allows the analysis of alternative splicing and an improved RNA velocity characterization enabling a better prediction of the dynamic processes occurring during mammalian development. Next, we compare the total transcriptomic profiles of the mouse gastruloids with our previously generated dataset looking at mouse embryo cells stages (E6.5-E9.5). Our detailed analysis reveals subtle differences and similarities between various embryonic cell types that were not previously known between gastruloids and mouse embryos. In addition, Vasa-seq facilitates the discovery of novel cell type markers for subtype identification based on non-coding RNA and allows an in-depth cell cycle analysis including the investigation of differential histone usage.

Funding Source: Dutch Research Council (NWO) - Talent Programme (VIDI) The Novo Nordisk Foundation Center for Stem Cell Medicine - Novo Nordisk Foundation grant (reNEW)
Keywords: Mouse Embryonic stem cells/Gastruloids, Single cell total RNA sequencing, Vasa sequencing

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PATTERNING FROM THE BOTTOM UP: HESC PATTERNING VIA SPATIALLY CONTROLLED STIMULATION FROM THE BASAL SIDE

Wyatt, Tom - *Wellcome - MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge*
Omais, Hassan - *Physico-Chimie Curie Lab, Institut Curie, Paris, France*
Sorre, Benoit - *Physico-Chimie Curie Lab, Institut Curie, Paris, France*
Bonavia, Sara - *Laboratoire Matière et Systèmes Complexes, Université Paris 7 Diderot, Paris, France*

Abstract: Pluripotent cells in the epiblast of the human embryo differentiate into a diversity of cell types during the process broadly known as gastrulation. These cellular decisions give rise to a precisely organised body plan and thus differentiation is necessarily linked to spatial patterning. Given the considerable inaccessibility of the human embryo to experimentation, we use human pluripotent stem cells (hPSCs) to model the epiblast in vitro and study patterning via the development of bioengineered approaches that enable quantitative control the environment. The morphogen BMP4 is crucial during gastrulation and it was recently discovered that BMP receptors are located baso-laterally in hPSC, as well as in the mouse epiblast, making those cells effectively insensitive to apically applied BMPs. We have thus developed a bioengineered system which stimulates hPSC monolayers on their basal side, whilst using microfluidics to quantitatively control the spatial profile of morphogen concentration. Using this system, we show that a parabolic shaped concentration gradient applied to the basal side is converted by hPSC to a classic Wolpertian 'French flag' pattern of 3 cell identities via a sigmoid-shaped signalling response which is stable in time. The high degree of control afforded by the system allows us to vary the shape of the profile and tissue independently, allowing us to establish that the patterning occurs via a true dose-response with respect to two robust concentration thresholds. In contrast, we find that a sharp (step-shaped) gradient, leads to altered patterning and the appearance of endodermal cell fates, as stimulated cells induce differentiation in their non-stimulated direct neighbours. By allowing control of two fundamental developmental patterning mechanisms, morphogen gradients and secondary inductions, we hope the tool will be of value to the community.

Keywords: gastrulation, patterning, morphogen gradient

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CULTURE OF PLURIPOTENT STEM CELLS AND DERIVATION OF ORGANOIDS USING DROPLET MICROFLUIDICS

Sart, Sébastien - *Genome and Genetics, Institut Pasteur, Paris, France*

Abstract: The formation of organoids recapitulates the processes of self-organization, symmetry breaking and tissue patterning that occur during the embryonic development. These morphogenetic events are driven by the creation of specific niches promoting the spatially controlled differentiation into specialized cell types. The biochemical confinement of differentiating PSCs into oil-isolated droplets has the potential to regulate the autocrine signaling that trigger tissue patterning during differentiation, which has not been demonstrated so far. In this presentation, I will first present a new droplet microfluidic platform that sustains the long-term culture of mouse embryonic stem cells (mESCs) at the undifferentiated state using reduced culture volumes. The platform is then used to derive several types of organoids from mESCs. In particular, I will demonstrate that the culture of mESCs into anchored microfluidic droplets enables the maturation of gastruloids and embryonic-like structures (ELSs), a type of organoids that recapitulate the early steps of the embryonic development. I show that ELSs display a unique head-trunk structure, which demonstrates high degree of similarity with the stage E8.5 of the mouse embryonic development (i.e. gene expression and structural organization, such as patterned somites and brain-like structures). As such, the differentiation of PSCs into microfluidics droplets provides a novel approach towards the derivation of more functional organoids, in view of tissue engineering and disease modeling applications.

Keywords: Gastruloids, Embryonic-like structures, microfluidics

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ANALYSIS OF A DEVELOPMENTAL ROLE OF RETINOIC ACID IN A GASTRULOID MODEL

Yildiz, Mehmet - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Dijkers, Pascale Francis - *Department of Anatomy & Embryology, Leiden University Medical Center & The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden, Netherlands*
Geijsen, Niels - *Department of Anatomy & Embryology, Leiden*

University Medical Center & The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Leiden, Netherlands

Abstract: Recent in vitro studies have shown that embryoid bodies undergo symmetry breaking, elongation and even self-organization into embryo-like structures termed gastruloids. This model for early embryogenesis allows examination of early axial body organisation in vitro, during which cells are committing to specific lineages as observed during embryonic development in vivo. Two well-known opposing signaling gradients, consisting of Wnt-FGF and retinoic acid, an active metabolite of vitamin A, have major roles in axial elongation and the organization of the body plan of the developing embryo. While retinoic acid has important roles during embryogenesis, however, the required levels and involvement in different stages of development are not clear. Wnt signaling in embryonic stem cells triggers mesoderm differentiation, while retinoic acid signaling drives neural differentiation. In this gastruloid model, we manipulated levels of vitamin A and retinoic acid in synthetic growth medium at different time points during gastruloid formation to examine effects on cell fate decision. Here, we will present data on the effects of temporal modulation of retinoic acid signaling in murine gastruloids. We examined the effect on axial body formation by analyzing the formation of the three germ layers and Hox gene patterning. Together, these data will provide insights into how modulating gradients in the embryo can influence elongation and cellular identity.

Funding Source: Funding: This project was supported, in part, by grants from the Novo Nordisk Foundation (NNF21CC0073729) and the ZonMW PSIDER program
Keywords: Early Embryonic Development, Gastruloids, Retinoic Acid Signaling

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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RECONSTRUCTING VASCULARIZED PRIMITIVE GUT TUBE WITH SPLANCHNIC MESODERM IN A DISH

Miao, Yifei - *Pulmonary Biology and Development Biology, Cincinnati Children's Hospital, Cincinnati, OH, USA*
Pastrana-Gomez, Victor - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Yu, Zhiyun - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Iwasawa, Kentaro - *Development Biology and CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Pek, Nicole - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Zorn, Aaron - *Development Biology and CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Wells, James - *Development Biology and CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Takebe, Takanori - *Development Biology and CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Whitsett, Jeffrey - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Guo, Minzhe - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Kotton, Darrell - *Center for Regenerative Medicine, Boston University and Boston Medical Center, Boston, MA, USA*
Gu, Mingxia - *Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Abstract: Lateral plate mesoderm (LPM)-derived splanchnic mesoderm (SM) forms the outer layer of the primitive gut tube. The proper arrangement of SM provides the appropriate signals pivotal for the normal development of embryonic gut tube and its derivatives. In tandem, the vasculature co-develops and intertwines with SM to support gut tube development, while reciprocally receiving molecular cues to adopt organ-specific fingerprints. This early developmental process is difficult to capture in human samples, thus, we aim to recapitulate this milestone event using an organoid system derived from iPSCs to better understand gut tube development and related diseases. Herein, we devised an optimized method where embryonic bodies are exposed to continual stimulation of Nodal signals and pulsatile induction by several other morphogens, such as Wnt, FGF2, and BMP4, essentially mimicking the early gastrulation stage. We observed the emergence of a mesendoderm-like spheroid composed of LPM and definitive endoderm. By modifying the differentiation cocktail, these miniature structures can be further regionalized into the primitive gut tube covered by FOXF1+ SM. Notably, vasculature marked by CD31 arose spontaneously, without the supplementation of pro-angiogenic factors. The regionalized and vascularized gut tube can then be embedded into 3D extracellular matrix and further specified into NKX2.1+ lung/thyroid, CDX2+/GATA4+ small intestinal, or CDX2+/SATB2 colonic progenitors. At this stage, exogenous angiogenic factors were introduced to further pattern the vasculature. Via in situ hybridization, we showed these endothelial cells adopted organ-specific gene signatures. Using this system, we sought to model a congenital disorder called Alveolar Capillary Dysplasia (ACD) caused by FOXF1 mutations. ACD is characterized by defects in pulmonary capillaries and impaired alveologenesis resulting in respiratory failure. We found SM formation was significantly hampered in vascularized gut tube organoids derived from iPSC harboring FOXF1 deletion. This was accompanied by reduced alveolar epithelial cells, indicating mesenchymal abnormalities underlie pulmonary defects seen in ACD. With this new organoid model, we now have a comprehensive platform to understand gut tube development and related disease in humans.

Funding Source: NHLBI PCTC
Keywords: Endothelial cell, Gut tube, Splanchnic mesoderm

TOPIC: EPITHELIAL_GUT

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PIEZO1 IN SMOOTH MUSCLE CELLS MODULATES SMALL BOWEL CONTRACILITY

Flores, Allison - *Pediatrics, University of California, Los Angeles, CA, USA*

Abstract: Piezo1 is a mechanosensitive, non-selective cation channel expressed in the gut's smooth muscle cells (SMCs). Yet, its role in intestinal muscularis cells (IMCs) has not been established. In vivo, ex vivo and in vitro methods were used to determine whether Piezo1 in SMCs is necessary for mediating intestinal motility and maintaining the crypt-villi axis during homeostatic and stretched conditions. We used a Piezo1/Myh11-ERT2/Cre-LoxP system to generate a tamoxifen-inducible-Piezo1 knockout (Piezo1ΔSMC) in the SMCs of the bowel. Piezo1ΔSMC mice had impaired growth and motility parameters. In vitro results show that at baseline, Piezo1ΔSMC leads to decreased frequency

and a dysrhythmic and asynchronous pattern of Ca2+ flux/contractility compared to Piezo1 WT IMCs, which is further reduced when acutely stretched. Complete ablation of Piezo1 in all cells of the IMCs using Piezo1 shRNA or chemical inhibitor leads to a complete cessation of Ca2+ flux/contractility. Similar findings were noted in human IMCs with chemical manipulation. In vivo results show that Piezo1ΔSMC leads to crypt and villi elongation and stem and goblet cell expansions identical to obstruction effects in Piezo1 WT mice. Our data suggest that Piezo1 in the SMCs of the muscularis is essential for the maintenance of regular and stretch-induced SMC contractions, gut motility and partly mediates epithelial, crypt, and stem cell expansion. These data improve our understanding of how mechanosensitive channels mediate these changes.

Keywords: smooth muscle, piezo1, muscularis

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MODELLING INFLAMMATORY BOWEL DISEASE IN HUMAN INTESTINAL ORGANOIDS USING A HIGH THROUGHPUT WORKFLOW

Brandenberg, Nathalie - *Business Development, SUN bioscience, Lausanne, Switzerland*
Ceroni, Camilla - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Clapes, Maria - *Business Development, SUN bioscience, Lausanne, Switzerland*
Hoehnel, Sylke - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Meyer, Marine - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Sirenko, Oksana - *Organoid Innovation Center, Molecular Devices, LLC, San Jose, CA, USA*
Storm, Robert - *Imaging, Molecular Devices, Berlin, Germany*

Abstract: Inflammatory bowel diseases (IBD) are characterized by chronic inflammations of the gastrointestinal track during which the intestinal mucosal barrier gets damaged. Until today, mice models have been used to unravel the complex interactions involved in IBD, yet they often fail to predict human responses. In recent years, organoids have emerged as a game-changing tool for disease modelling and drug screening. These organoids are three-dimensional, miniaturized and simplified versions of an organ that mimic some of the key features of the native tissue in vitro. Traditional organoid culture methods consist of embedding these structures in solidified extracellular matrix (ECM) thus introducing an intrinsic lack of reproducibility and creating highly heterogeneous organoid populations. To overcome these challenges, we used Gri3D®, an innovative hydrogel-based ultra-dense U-bottom shaped microcavity array platform. Gri3D® enables the generation of a single organoid in each microcavity in suspension-like conditions, without a solid ECM, allowing organoid cultures standardization. Combined with a high-content imaging ImageXpress Micro Confocal system, organoids were live-monitored over time to track key IBD related phenotypes at a single-organoid level. We report the induction of intestinal inflammation on healthy human rectal organoids using pro-inflammatory cytokines (TNF-α and Il-1β). Upon treatment, the epithelial barrier was disrupted and further assessed by immunostaining of tight junctions. Interestingly, treated organoids show slower growth rate and decreased budding capacity. We demonstrate here the use of Gri3D® as a robust and high-throughput in vitro platform for human GI organoid-based IBD modelling.

Funding Source: All presented work was self-funded in a collaborative framework between SUN bioscience SA and Molecular Devices.
Keywords: Organoids, Screening, IBD

TOPIC: EPITHELIAL_LUNG

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STUDY OF GENETIC VARIANTS INFLUENCING CYSTIC FIBROSIS LUNG DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Pinte, Laetitia - *Stem Cell, Cambridge University, Cambridge, UK*
Vila-Gonzalez, Marta - *Stem Cell, Cambridge University, Cambridge, UK*
Causa, Erika - *Physics, Cambridge University, Cambridge, UK*
Fradique, Ricardo - *Physics, Cambridge University, Cambridge, UK*
Floto, Andres - *Medicine, Cambridge University, Cambridge, UK*
Cicuta, Pietro - *Physics, Cambridge University, Cambridge, UK*
Vallier, Ludovic - *Stem Cell, Cambridge University, Cambridge, UK*

Abstract: Cystic Fibrosis (CF) is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene coding for a chloride- and bicarbonate-ion channel. Deficient or dysfunctional CFTR changes ion composition resulting in abnormal mucus, affecting transport and fluid's homeostasis in multiple epithelia. Lung disease is responsible for the majority of patients' morbimortality. Intriguingly, more than half of its phenotype is not due to CFTR mutations, but to the environment and genetic modifiers. Genome-Wide Association Studies (GWAS) have identified Single Nucleotide Polymorphisms (SNPs) that could be genetic modifiers. One of the regions outlined (11q13) is located close by an epithelium-specific gene named ETS Homologous Factor (EHF). Here, we hypothesized EHF may be impacted by these variants and may play a crucial role in the airway epithelium. We used human-induced Pluripotent Stem Cells (hiPSC)- derived Airway Epithelial Cells (AECs). Our model generates a polarized pseudostratified epithelium containing basal cells (TP63, NGFR), goblet cells (MUC5AC, MUC5B), club cells (SCBG1A1, SCBG3A2), and ciliated cells (FOXJ1, ACTUB) with motile cilia. In our model, EHF was highly expressed in mature AEC, suggesting an important function for this transcription factor in the airway epithelium. To understand its role, we knocked out EHF in hiPSCs using CRISPR/Cas9. Corresponding EHF-/- AECs expressed lower secretory cells and mature basal cell markers. Markers of ciliated cells were unchanged, but cilia motility and synchronism were decreased when assessed by microscopy. Transepithelial electrical resistance was significantly higher in EHF-/- iPSC-derived AECs while cell migration was not significantly impaired by wound scratch assay. Importantly, preliminary data indicated that HIF-dependant response to hypoxia was decreased in EHF-/- cells. Taken together, these results suggest a key function for EHF in the functioning of lung epithelium, especially in stress-response. To conclude, EHF is involved in the regulation of cilia motility and response to stress. These findings could pave the way to finding new approaches for personalized treatments.

Funding Source: Cystic Fibrosis Trust
Keywords: Cystic Fibrosis lung disease, Genetic modifiers, EHF

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LUNG EPITHELIAL ORGANOIDS DERIVED FROM MUTANT AND ISOGENIC CFTR IPSCS AS CYSTIC FIBROSIS DISEASE MODELS OF INFLAMMATORY RESPONSES

Beltran, Adriana S. - *Genetics and Pharmacology, University of North Carolina at Chapel Hill, NC, USA*
Avendaño-Félix, Mariana - *Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacan, Mexico*
Beltran, Alvaro - *Neuroscience Center, University of North Carolina at Chapel Hill, NC, USA*
Marquez, Ariana - *Human Pluripotent cell core, University of North Carolina at Chapel Hill, NC, USA*
Molina, Sarahi - *Human Pluripotent Cell Core, University of North Carolina at Chapel Hill, NC, USA*
Olivares, Felix - *Pharmacology, University of North Carolina at Chapel Hill, NC, USA*
Ramos-Payán, Rosalío - *Universidad Autónoma de Sinaloa, Facultad de Ciencias Químico Biológicas, Sinaloa, Mexico*

Abstract: Cystic fibrosis (CF) is a genetic disorder in which thick mucus secretions compromise the function of multiple organs. Once CF disease starts, inflammatory processes play important roles in disease progression. However, the function of CFTR mutations in the establishment of cellular inflammatory stress during disease initiation remains controversial. Studies exploring this question are often conducted either in cell lines (+/- CFTR) or on cells derived from CF or non-CF patients, where potential confounders, clonal differences, variability between human subjects, and epigenetic changes related to lifelong inflammation, rather than CFTR mutations, might lead to interpretive incongruities. To study the CFTR response in inflammatory states, we generated four isogenic CFTR variants (F508del, F508del-I507; ATC➔ATT, I507; ATC➔ATT, and CFTR knockout (KO) using the CRISPR/Cas9 system. Mutant CFTR and isogenic iPSCs were differentiated into lung epithelial organoid cultures and used to assess the efficacy of CF modulator treatments and to characterize the inflammatory response. First, we performed swelling assays with forskolin and compared the efficacy of CF modulator treatments in the CFTR lung epithelial organoid cultures versus isogenic, then we assessed the percentage of rescued CFTR responses. Second, we assessed cytokine production in undifferentiated vs. differentiated cells at baseline and after stimulation. The forskolin-induced swelling assay demonstrated reduced activity of CFTR in the point mutations, isogenic CFTR epithelial organoids compared to controls, while the KO did not swell. Furthermore, the wildtype iPSCs do not express CFTR or inflammatory cytokines such as IL8, but differentiation to CFTR-lung epithelial organoids showed increased levels of IL-8. We are currently assessing IL-1β and supernatant from mucopurulent material (SMM) effect on mucin secretion and other inflammatory genes. This study offers a relevant endogenously expressing model system with an isogenic background to study mechanistic details of the molecular nature of CFTR's inflammatory responses.

Keywords: CFTR, lung epithelial organoid, CRISPR

TOPIC: EPITHELIAL_SKIN

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KRT15 AS A PUTATIVE MARKER FOR CANCER STEM CELLS IN ESOPHAGEAL CANCER

Douchin, Julie - *Immunology and Cellular Biology, Université de Sherbrooke, Sherbrooke, PQ, Canada*
Giroux, Véronique - *Immunology and Cellular Biology, Université de Sherbrooke, Sherbrooke, QC, Canada*

Abstract: Esophageal cancer has a 5-year survival rate of only 15% worldwide. This high mortality rate is partly due to treatment resistance occurring in 20 to 40% of patients. This resistance to standard treatment is attributed to the persistence of cancer stem cells (CSC). CSCs are described as normal stem cells who transition to CSCs during tumorigenesis. They are multipotent, have self-renewal capacity and display low sensitivity to chemotherapy and radiation. Our previous work identified Krt15+ cells as the main stem cell population in the esophageal epithelium, suggesting Krt15+ cells as potential CSCs in esophageal cancer. Therefore, this project aims to determine if Krt15+ tumor cells act as CSCs in esophageal cancer. To study Krt15+ cells behavior during esophageal cancer progression, we induced esophageal cancer in Krt15-CrePR1;R26mT/mG mice by adding 4-Nitroquinoline-1-oxide (4NQO) carcinogen to their drinking water. When tumors were developed, Cre recombination was performed through PR agonist administration to induce GFP expression specifically in Krt15+ cells leading to lineage tracing. Krt15+ and Krt15- esophageal tumor cells were FACS-sorted and used to establish Krt15+ and Krt15- derived tumoroids. First, we used p63 as a marker of basal (undifferentiated) cells and K13 as a differentiation marker in immunofluorescence. By studying basal cell localization in the organoids as well as using the ratio of p63 positive cells by K13 positive cells, we found that Krt15+ cells form better organized organoids compared to Krt15- cells. Furthermore, Krt15+ tumoroids display hyperplasia in their basal layer suggesting a more advanced phenotype. Interestingly, we observed an increase of CSC-like cells within the Krt15+ tumoroids when compared to Krt15- tumoroids using flow cytometry against CD44/CD24, well-known CSC markers. Moreover, preliminary data suggest that Krt15+ organoids express higher levels of Smoc2 and Lrig1 mRNA, two stem cell markers, compared to Krt15- organoids. To conclude, our mouse-derived organoid model revealed that Krt15+ cells could act as CSCs in esophageal cancer. We aim to highlight Krt15+ cells potential as diagnostic or prognostic markers and put forward new targets to overcome treatment resistance in esophageal cancer patients.

Funding Source: Canadian Association of Gastroenterology (CAG) Canadian Institutes of Health Research Université de Sherbrooke Fondation canadienne pour l'innovation NSERC Chaires de recherche du Canada
Keywords: esophageal cancer, lineage tracing, cancer stem cells

TOPIC: EYE AND RETINA

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USING HUMAN CONJUNCTIVA ORGANOIDS TO STUDY OCULAR SURFACE HOMEOSTASIS AND DISEASE

Bannier-Hélaouët, Marie - *Hubrecht Institute, Utrecht, Netherlands*
Korving, Jeroen - *Hubrecht Institute, Utrecht, Netherlands*
Lamers, Mart - *Viroscience, Erasmus MC, Rotterdam, Netherlands*
Begthel, Harry - *Hubrecht Institute, Utrecht, Netherlands*
López Iglesias, Carmen - *M4I, Maastricht University, Maastricht, Netherlands*
van de wetering, Willine - *M4I, Maastricht university, Utrecht, Netherlands*
Büll, Christian - *Hubrecht Institute, Utrecht, Netherlands*
Yawata, Nobuyo - *Graduate School of Medical Sciences, Ocular Imaging and Pathology, Kyushu University, Kyushu, Japan*
Lapointe, Vanessa - *MERLN, Maastricht University, Maastricht, Netherlands*
Dickman, Mor - *Ophtalmology, Maastricht UMC, Maastricht, Netherlands*
Kalmann, Rachel - *Ophtalmology, UMC Utrecht, Utrecht, Netherlands*
Imhof, Saskia - *Ophtalmology, UMC Utrecht, Utrecht, Netherlands*
Peters, Peter - *M4I, Maastricht University, Maastricht, Netherlands*
Haagmans, Bart - *Viroscience, Erasmus MC, Rotterdam, Netherlands*
Clevers, Hans - *Hubrecht Institute, Utrecht, Netherlands*

Abstract: The conjunctival epithelium, which lines the inside of the eyelids and the sclera (white of the eye), is essential to maintain ocular health and corneal transparency. It consists of two main differentiated cell types: mucus-producing Goblet cells and keratinocytes of unknown function. The conjunctiva is the first line of defense of the eye and can be infected by numerous viruses, causing impaired vision. Yet, reliable models of human conjunctival homeostasis and disease are currently lacking. We have developed organoids derived from mouse and human conjunctiva that contain all conjunctival cell types. Using single-cell RNA sequencing, we showed that conjunctival keratinocytes expressed a broad range of anti-microbial peptides. Conjunctiva organoids secreted mucus and revealed the previously unknown ability of the conjunctival keratinocytes to secrete anti-microbial peptides and, thus, to participate in the protection of the eye from infections. We then modelled viral conjunctivitis in a dish. Conjunctival cultures were successfully infected by Herpes Simplex Virus 1 (HSV1), human Adenovirus 8 (hAdV8) and SARS-CoV2. HSV1 infection was reversed by acyclovir addition, while hAdV8 infection, for which there is currently no drug, was inhibited by cidofovir. Lastly, conjunctival organoids engrafted orthotopically and now constitute a potential source of tissue for human transplantations. Together, human conjunctiva organoid cultures enable the study of conjunctival physiology, drug development and cell therapy.

Keywords: Conjunctiva, Organoids, Viral infections

TOPIC: GERMLINE

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GENERATION OF A WNT MIMETIC USING C. DIFF. TOXIN B

Kenner, Nathaniel - *Biology, California State University, San Marcos, Fallbrook, CA, USA*

Abstract: Wnt proteins are a class of secreted proteins with a wide range of activities in cell fate, specification, differentiation, and proliferation, and induce these effects when bound to two Wnt surface receptor proteins: Frizzled and LRP6. However, since Wnt proteins are hydrophobic and are therefore difficult to purify and study, the use of engineered Wnt agonists, referred to as Wnt mimetics, allows researchers to continue studying without the need of lengthy Wnt purification protocols. Clostridium difficile is a bacterium that secretes a toxin known as Toxin B (TcdB) that binds to FZD receptors and disrupts cellular architecture of the intestinal epithelium. The Frizzled Binding Domain (FBD) of TcdB targets most, if not all FZDs, thus providing an opportunity to develop a broad specificity Wnt mimetic. We hypothesized that using the FBD of TcdB in tandem with an LRP6 binding site would create a Wnt mimetic that could heterodimerize any FZD with LRP6 and thereby activate downstream signaling. We designed a series of Wnt mimetics comprised of various portions of the FBD linked to an LRP6-specific single chain variable fragment (scFv). For purification purposes we appended the IgG1 constant region, as well as a signal sequence to ensure secretion. Plasmids encoding these Wnt mimetics have been transfected into CHO cells and expression has been verified by immunoblotting. We found that several versions of these proteins are produced by CHO cells, and we are currently investigating whether these proteins are secreted into the extracellular environment. A luciferase-based Wnt reporter assay, called TOP-Flash, was performed to test signaling activity. Two versions of these novel Wnt mimetics produced an increase in Wnt activity. Production of stable clonal cell lines expressing these Wnt mimetics is ongoing. These proteins show promise as Wnt mimetics and preliminary testing indicates that these proteins can be produced in a state that stimulates the Wnt pathway.

Keywords: Wnt protiens, cell fate, Frizzled

TOPIC: HEMATOPOIETIC SYSTEM

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INVESTIGATING THE MECHANISM OF NOTCH-SIGNALING IN ADULT EXTRAMEDULLARY HEMATOPOIESIS

Bandara, Thilinie - *Department of Pharmacology, University of Illinois at Chicago, IL, USA*
Shao, Lijian - *Department of Pharmacology, University of Illinois at Chicago, IL, USA*
Sottoriva, Kilian - *Pharmacology, University of Illinois at Chicago, IL, USA*
Paik, Na Yoon - *Pharmacology, University of Illinois at Chicago, IL, USA*
Pajcini, Kostandin - *Pharmacology, University of Illinois at Chicago, IL, USA*

Abstract: Developing hematopoietic stem cells (HSC) in the fetal liver undergo massive expansion and self-renewal. Fetal HSCs

migrate to the bone marrow (BM) niche towards birth and remain there during adulthood. Extramedullary hematopoiesis (EMH), or hematopoiesis outside of the BM, predominantly occurs during fetal liver development or when the adult BM niche ceases to be a site for functional hematopoietic stem cells and progenitors. The mechanism by which the adult liver becomes reactivated as a site of EMH for itinerant HSCs is unknown. Furthermore, myelofibrosis and cytopenia are hematopoietic disorders driven by EMH with unknown causes. Our project is investigating EMH in the adult liver by inducing acute hemolytic anemia in adult mice. Following induction of EMH in wild-type mice, we found that hematopoietic progenitors decrease in Phenylhydrazine (PHZ)-treated BM and increase in liver and spleen populations. Our work establishes that HSCs homing to the adult liver can serially reconstitute lethally irradiated mice suggesting that the adult liver serves as a potential reservoir for long-term HSCs in the presence of a damaged BM niche. In pursuit of a mechanism, we focused on Notch-signaling which is an evolutionarily conserved pathway that has been shown to play a critical role in HSC emergence and fetal liver development. When comparing WT liver donors to Notch-deficient (Notch1-ΔTAD) liver donors, our hematopoietic transplants reveal that Notch1-ΔTAD donor progenitors fail to efficiently reconstitute irradiated recipients. After PHZ treatment, we found the Notch1 receptor decreases in BM progenitors, but Notch2 is induced in the liver progenitors. We characterized transcriptomic profiles of HSCs in resting bone marrow and EMH-activated BM as well as adult liver to determine the Notch target genes essential for EMH hematopoiesis. Our findings indicate that Notch signaling is a required pathway that maintains functional long-term HSCs during EMH.

Funding Source: T32 NIH Training Grant
Keywords: Hematopoiesis, Notch, Stem Cells

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CELL AND CONTEXT SPECIFIC DEREGULATION OF INNATE IMMUNE DEAMINASES IN STEM AND PROGENITOR POPULATIONS IN MYELOPROLIFERATIVE NEOPLASMS

Isquith, Jane M. - *School of Medicine, University of California, San Diego, CA, USA*
Pham, Jessica - *School of Medicine, University of California, San Diego, CA, USA*
Mark, Adam - *School of Medicine, University of California, San Diego, CA, USA*
Donohoe, Mary - *School of Medicine, University of California, San Diego, CA, USA*
Whisenant, Thomas - *School of Medicine, University of California, San Diego, CA, USA*
Jamieson, Catriona - *School of Medicine, University of California, San Diego, CA, USA*

Abstract: Inflammatory cytokine responsive APOBEC3 cytidine deaminases have been studied extensively with regard to innate immunity and more recently during cancer evolution. However, the mechanisms by which the APOBEC3 enzymes promote cancer initiation and progression in the malignant microenvironment remains to be investigated, especially in hematopoietic malignancies. Through whole genome and whole transcriptome sequencing analyses of MPN patient samples FACS sorted into stem and progenitor populations, we have found a cell type and context specific nature of these enzymes, notably the upregulation of APOBEC3C (A3C) in the high-risk Myelofibrosis (MF) stem cell population as compared to normal aged counterparts. Through lentiviral overexpression of each APOBEC3 enzyme, we can now study the effects of changes in APOBEC3 in relation to the known changes in expression seen in many cancers, focusing on the upregulation of A3C in sorted stem and progenitor cell populations. Using these techniques, we have identified novel RNA and DNA editing targets, as well as differential gene expression patterns of each APOBEC3 in normal CD34+ cord blood and aged normal bone marrow. Gene set enrichment analysis (GSEA) performed on this dataset has exposed numerous deregulated pathways brought on by exaggerated levels of APOBEC3, including changes in splicing pathways. In addition, novel identification of the relationship between A3C and ADAR1, another innate immune deaminase, has important implications in initiation and prognosis of MPNs. Both A3C and ADAR1 transcript levels are elevated in high-risk MF stem cells, and co-immunoprecipitation studies reveal a direct binding of the enzymes. This novel connection, as well as the role of A3C in initiation and progression of hematopoietic malignancies will continued to be studied using this system to elucidate effects on proliferation, differentiation, self-renewal, and changes to the cell cycle, as well as the intricacies of these changes in normal and malignant stem cell populations.

Keywords: deaminase, hematopoetic, cancer stem cell

TOPIC: IMMUNE SYSTEM

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WISKOTT-ALDRICH SYNDROME PROTEIN REGULATES NUCLEOLUS STRUCTURE AND RNA POLYMERASE I TRANSCRIPTION

Zhou, Xuan - *BESE, King Abdullah University of Science and Technology (KAUST), Jeddah, Saudi Arabia*
Yuan, Baolei - *BESE, KAUST, Thuwal, Saudi Arabia*
Wang, Mengge - *BESE, KAUST, Thuwal, Saudi Arabia*
Ramos, Gerardo - *BESE, KAUST, Thuwal, Saudi Arabia*
Li, Mo - *BESE, KAUST, Thuwal, Saudi Arabia*

Abstract: Wiskott–Aldrich syndrome (WAS) is a primary immunodeficiency disorder in the hematopoietic system caused by mutations in the WAS protein (WASP). The clinical features are characterized by thrombocytopenia, eczema, recurrent infections, autoimmunity, and early death. WASP is known for stimulating actin polymerization through its VCA domain, and the complex phenotypes of WAS are often associated with defects in actin polymerization. However, transfecting the VCA-lacking WASP mutant into WASnull T cells can rescue gene activation defects, indicating additional functions of WASP also contribute to the disease. Meanwhile, studies show that WASP is located in the nucleus and regulates transcription by RNA polymerase II in T cells. Whether WASP performs other nuclear roles in different immune cells remains unexplored. To address this question, we generate isogenic macrophage cells derived from WASP knockout human pluripotent stem cells. Our preliminary data show that WASP interacts with nucleolar proteins and regulates nucleolar organization. Because the nucleolus is the site of ribosome biogenesis, we further evaluate the effect of WASP on ribosomal RNA (rRNA) transcription by RNA polymerase I. Our results provide evidence that WASP deficiency significantly reduces rRNA transcription. We will present results of ongoing experiments, including RNA-seq, proteomics, fluorescence recovery after photobleaching, etc., that attempt to address the following questions: (i) Is the lower rRNA transcription in WASP-deficient macrophage cells associated with lower rates of protein synthesis? (ii) Does WASP

deficiency lead to changes in the liquid-liquid phase separation property of the nucleolus and its constituents? (iii) What are the functional consequences of the nucleolar defects in WASP-deficient macrophages? Overall, our macrophage model could provide insights into novel mechanisms of WASP in immune function through its nuclear role.

Funding Source: The research of the Li laboratory was supported by KAUST Office of Sponsored Research (OSR), under award numbers BAS/1/1080-01
Keywords: Wiskott–Aldrich syndrome protein, isogenic macrophage cells, nucleolus structure

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HETEROGENEITY AND FUNCTION OF CONVENTIONAL DENDRITIC CELLS DIFFERENTIATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Ito, Fumito - *Department of Surgery, University of Southern California, Los Angeles, CA, USA*
Long, Mark - *Department of Biostatistics & Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Kajihara, Ryutaro - *Center for Immunotherapy, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Matsueda, Satoko - *Center for Immunotherapy, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Oba, Takaaki - *Center for Immunotherapy, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Kanehira, Kazunori - *Department of Pathology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Liu, Song - *Department of Biostatistics & Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Makino, Kenichi - *Center for Immunotherapy, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*

Abstract: Dendritic cells (DCs) are a diverse population of specialized antigen presenting cells that link innate and adaptive immunity, and crucial in the induction of immune responses to pathogens and tumors. Accumulating evidence suggests that conventional type 1 DCs (cDC1) excel in cross-presentation of exogenous antigens on MHC-I molecules, and induction of antitumor CD8+ T cell immunity; however, obtaining large numbers of cDC1s is difficult. The use of reprogramming and differentiation techniques could overcome this limitation; however, the type and developmental pathway of human induced pluripotent stem cell (iPSC)-derived DC remain elusive. Here, we performed single-cell profiling and functional characterization of DC differentiated from human iPSC on OP9 feeder cells and OP9 expressing the Notch ligand delta-like 1 (OP9-DL1). Human iPSC were established from monocytes and T cells. Single-cell RNA sequencing of human iPSC-derived HLA-DR+ cells identified the substantial heterogeneity of conventional DCs including CD141+XCR1+CLEC9A+ cells (cDC1), CLEC4AhiCLEC10A–CD1c+ cells (cDC2A), CLEC4AloCLEC10A+CD1c+ cells (cDC2B), CD163–CD5+CD1c+ cells (CD5+cDC2), and AXL+SIGLEC6+ cells (AS-DC). Of these, generation of cDC1, cDC2, and AS-DC was Notch-dependent while DC3 were found mainly on OP9 feeder cells. Plasmacytoid DCs were not differentiated from iPSCs on either OP9 or OP9-DL1 cells. Both monocyte- and T-cell-derived human iPSC gave rise to cDC1. We found that pHrodo zymosam particles were efficiently phagocytosed by human iPSC-derived cells, and iPSC-derived cDC1, cDC2A, cDC2B and DC3 have comparable phagocytic ability. Human iPSC-DC expanded CD8+ and CD4+ T cells from the same donor in the presence of MHC class I or II peptides specific to various viruses and IL-2 in vitro, respectively with effective downregulation of CD62L and CD45RA and upregulation of CD25 on T cells. Furthermore, human iPSC-derived cDC1, cDC2A, cDC2B and DC3 produced IL-12 and TNF-α upon stimulation with TLR agonists, poly(I:C), R848, LPS and CpG. Taken together, these results revealed a critical role of Notch signaling in differentiating conventional DC, and provided insights into the future development of personalized treatment with unlimited numbers of autologous cDC from human iPSCs in the clinic.

Funding Source: Melanoma Research Alliance and the Sarcoma Foundation of America (F. Ito), Uehara Memorial Foundation (T. Oba), and NIH/NCI grant, U24CA232979 (M. Long and S. Liu), K08CA197966 and R01CA255240-01A1 (F. Ito) and P30CA016056 (Roswell)
Keywords: dendritic cells, hematopoiesis, Notch signaling

TOPIC: KIDNEY

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HUMAN IPS CELLS CARRYING SMAD2 GENETIC MUTATIONS EXHIBIT ABERRANT DIFFERENTIATION INTO KIDNEY PODOCYTES

Bhattacharya, Rohan - *Biomedical Engineering Center for Biomolecular and Tissue Engineering, Duke University, Durham, NC, USA*
Ward, Tarsha - *Genetics, Harvard Medical School, Boston, MA, USA*
Kalejaiye, Titilola - *Biomedical Engineering, Duke University, Durham, NC, USA*
Mehta, Nalini - *Biomedical Engineering, Duke University, Durham, NC, USA*
Bonner, Makenzie - *Center for Biomolecular and Tissue Engineering, Developmental and Stem Cell Biology, Cell Biology, Duke University, Durham, NC, USA*
Leeman, Sophia - *Biomedical Engineering, Duke University, Durham, NC, USA*
Siedman, Christine - *Genetics, Medicine (Cardiovascular Division), Harvard Medical School, Brigham and Women's Hospital, Howard Hughes Medical Institute, Boston, MA, USA*
Siedman, Jonathan - *Genetics, Harvard Medical School, Boston, MA, USA*
Musah, Samira - *Biomedical Engineering, Center for Biomolecular and Tissue Engineering, Developmental and Stem Cell Biology, Cell Biology, Medicine (Nephrology Division), Duke University, Durham, NC, USA*

Abstract: Early cardiovascular and renal development involve extensive cell lineage diversification and tissue patterning through distinct and shared molecular signaling networks. Recent clinical observations suggest that anomalies in cardiovascular development augment the risks for kidney disease however, this niche of the field is largely undeveloped. This cardio-renal relationship can be exacerbated by variants of the SMAD2 gene. Despite its clinical importance in organ patterning, the impact of SMAD2 mutations during embryonic kidney development remains unexplored. Here, we used CRISPR/Cas9 to introduce two homozygous mutations in the SMAD2 gene in a human-induced pluripotent stem (iPS) cell line. Our results show that abrogation of SMAD2 causes biased mesoderm lineage commitment characterized by altered expression of T, GSC, and CDX2. By harnessing the developmental programming of human iPS cells, we differentiated mesoderm cells into nephrogenic intermediate mesoderm (IM) cells via tem-

poral control of WNT-BMP7 signaling pathways. Interestingly, the differentiating mutant mesoderm cells display myofibroblast-like phenotype and over-express TWIST1 and SNAI1 proteins typically associated with epithelial to mesenchymal-like transition (EMT). Immunoblotting showed that some of these differentiating IM cells also express RIPK3 and its downstream target, MLKL tetramers, indicating necroptosis-mediated cell death. Upon further lineage specification toward the differentiated kidney podocytes, the SMAD2 variants failed to develop morphological features such as the arborized cellular architecture and foot processes observed in the wild-type cells. The resulting mutant podocytes demonstrated mislocalization and altered expression of lineage (Neph, Pod, Synpo) and EMT markers along with expression of αSMA, and NKD2 markers which are associated with fibrosis. Our results indicate that SMAD2 variants of isogenic human iPS cell-derived podocytes exhibit a dysregulated TGF-ß/SMAD2 signaling axis causing aberrant changes in transcriptomic and proteomic profiles, consequently dysregulating cell fate decisions. This work could help illuminate molecular mechanisms of altered nephrogenesis in patients with congenital heart disease, including those resulting from SMAD2 mutations.

Funding Source: NIH T32 Grant T32GM800555, Whitehead Scholarship, George O'Brien Kidney Pilot Grant (P30 DK081943), MEDxPilot Grant, Burroughs Wellcome Fund PDEP Career Transition Ad Hoc Award, Duke Incubation Fund, Genentech Research Award
Keywords: Isogenic human iPS cells, SMAD2 mutation, Kidney podocytes

TOPIC: LIVER

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MODELING THROMBOTIC MICROANGIOPATHY USING HUMAN PSC-DERIVED LIVER SINUSOIDAL ENDOTHELIAL CELLS

Kawamura, Shuntaro - *Institute of Research, Tokyo medical & dental university, Bunkyo, Japan*
Yoneyama, Yosuke - *Institute of Research, Tokyo Medical and Dental University (TMDU), Bunkyo, Japan*
Saiki, Norikazu - *Institute of Research, Tokyo Medical and Dental University (TMDU), Bunkyo, Japan*
Maezawa, Mari - *Institute of Research, Tokyo Medical and Dental University (TMDU), Bunkyo, Japan*
Okada, Noriki - *Department of Gastroenterological, General and Transplant Surgery, Jichi Medical University, Shimotsuke, Japan*
Sanada, Yukihiro - *Department of Gastroenterological, General and Transplant Surgery, Jichi Medical University, Shimotsuke, Japan*
Onishi, Yasuharu - *Department of Gastroenterological, General and Transplant Surgery, Jichi Medical University, Shimotsuke, Japan*
Sakuma, Yasuharu - *Department of Gastroenterological, General and Transplant Surgery, Jichi Medical University, Shimotsuke, Japan*
Takebe, Takanori - *Institute of Research, Tokyo Medical and Dental University (TMDU), Bunkyo, Japan*

Abstract: Thrombotic microangiopathy (TMA) is a lethal complication found in the solid organ transplantation, characterized by endothelial injury, microvascular thrombosis, and multi-organ injury. Anti-thymocyte globulin (ATG), a second-line immunosuppressant used in the liver transplantation, reportedly triggers platelet activation and likely associates with TMA, however, precise mechanism is ill-defined. Reproducing the pathogenesis in conventional laboratory animals is almost impossible due to a lack of antibody cross-reactivity ushering in the human-specific model development for interrogating mechanisms of TMA. Here, we established an in vitro TMA model that recapitulates the sequential thrombotic events involving complement cascade activation and microthrombus formation in the liver sinusoid with human induced pluripotent stem cells (iPSCs). We first directly differentiated iPSCs into liver-specific sinusoidal endothelial-like cells (iSECs) that are able to produce a hemostatic factor von Willebrand factor (vWF). Over 80% of iSECs expressed CD32b, the liver sinusoidal endothelial cell marker, and vWF. Treating iSEC with 0.5 mg/ml of ATG and human serum resulted in the formation of three times more complement activation products, membrane attack complex (MAC), on the cell surface than with normal rabbit IgG (rIgG). The addition of human platelets resulted in the formation of microthrombotic structures that consisted of aggregated platelets on vWF multimers. The number of microthrombi was increased by more than 30% with ATG 0.1 mg/ml and twofold with ATG 0.5 mg/ml compared with rIgG. vWF multimers had a maximum length of less than 50 µm with rIgG, but more than 200 µm with ATG 0.5 mg/ml. Pharmacological inhibitor that blocked alternative complement pathway reduced the maximum length of vWF multimers to less than 100 µm and inhibited MAC formation by more than 90%, whereas the number of microthrombi was only reduced by about 20%. Given the effects of complement activation and pharmacological inhibition on microthrombus formation can be assessed, this model of microvessel thrombosis will allow the study of the liver sinusoid-specific interactions between the complement system and platelets and the development of TMA therapies.

Funding Source: Support for Pioneering Research Initiated by the Next Generation
Keywords: Pluripotent stem cell, Liver sinusoid, Thrombotic microangiopathy

TOPIC: MUSCULOSKELETAL

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NF1 REGULATES WNT SIGNALING IN BONE MARROW SKELETAL STEM CELLS TO MAINTAIN THE ADULT SKELETON

Khalid, Aysha B. - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Paria, Nandina - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite Hospital for Children, Dallas, TX, USA*
Shen, Bo - *Children's Research Institute, UT Southwestern Medical Center, Dallas, TX, USA*
Oxendine, Ila - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite Hospital for Children, Dallas, TX, USA*
Cornelia, Reuel - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite Hospital for Children, Dallas, TX, USA*
Wise, Carol - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite Hospital for Children, Dallas, TX, USA*
Rios, Jonathan - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite Hospital for Children, Dallas, TX, USA*

Abstract: Neurofibromatosis Type 1 (NF1) is a tumor predisposition syndrome with pleiotropic skeletal manifestations, including generalized osteopenia/osteoporosis and bone fractures. Conditional mouse models show Nf1 is required in skeletal cartilage during embryonic development; however, it remains unclear whether Nf1 is required to maintain the adult skeleton. Here, we engineered LepR-cre;Nf1f/- mice (Nf1LepR) to conditionally delete Nf1 in adult (>2 months) bone marrow skeletal stem cells (SSCs). At 4 months of age, Nf1LepR mice were indistinguishable from controls. However, mCT analysis of femur and tibia showed significantly reduced trabecular bone volume and thickness, while the cortical bone showed increased porosity and decreased thickness in Nf1LepR mice compared to controls. We then tested osteogenic differentiation of SSCs from Nf1LepR and control mice. Osteogenic differentiation was significantly impaired in SSCs from Nf1LepR mice, which was associated with a lack of Wnt pathway activation. We then treated control and Nf1LepR SSCs with Osteolectin, a Wnt agonist expressed by SSCs that, via its receptor Integrin alpha 11 (Itga11), is required for maintenance of the adult skeleton. Compared to SSCs from control mice, Osteolectin did not rescue osteogenesis or Wnt pathway activation in SSCs from Nf1LepR mice. The reduced osteogenic differentiation and Wnt pathway activation of Nf1LepR SSCs was also associated with significantly reduced expression of Itga11. Taken together, our results show that Nf1 is essential for bone marrow SSCs to maintain the adult skeleton, possibly by regulating Wnt signaling during osteogenesis, and that loss of Nf1 in LepR+ SSCs in mice produces skeletal disease recapitulating the phenotype in human NF Type 1.

Funding Source: Pediatric Orthopaedic Society of North America, Texas Neurofibromatosis Foundation, Department of Defense, National Cancer Institute
Keywords: Neurofibromatosis, skeletal stem cell, WNT

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CHARACTERIZATION OF EARLY-ONSET FINGER OSTEOARTHRITIS-LIKE CONDITION USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

Rim, Yeri Alice - *Catholic Induced Pluripotent Stem Cell Research Center, Seoul St Mary's Hospital, Seoul, Korea*
Ju, Ji Hyeon - *Department of rheumatology, Catholic University of Korea, Seoul, Korea*
Nam, Yoojun - *Research & Development Department, YIPSCELL, Seoul, Korea*

Abstract: Early osteoarthritis (OA)-like symptoms are difficult to study owing to the lack of disease samples and animal models. In this study, we generated induced pluripotent stem cell (iPSC) lines from a patient with a radiographic early-onset finger osteoarthritis (efOA)-like condition in the distal interphalangeal joint and her healthy sibling. We differentiated those cells with similar genetic backgrounds into chondrogenic pellets (CPs) to confirm efOA. CPs generated from efOA-hiPSCs (efOA-CPs) showed lower levels of COL2A1, which is a key marker of hyaline cartilage after complete differentiation, for 21 days. Increase in pellet size and vacuole-like morphologies within the pellets were observed in the efOA-CPs. To analyze the changes occurred during the development of vacuole-like morphology and the increase in pellet size in efOA-CPs, we analyzed the expression of OA-related markers on day 7 of differentiation and showed an increase in the levels of COL1A1, RUNX2, VEGFA, and AQP1 in efOA-CPs. IL-6, MMP1, and MMP10 levels were also increased in the efOA-CPs. Taken together, we present proof-of-concept regarding disease modeling of a unique patient who showed OA-like symptoms.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT, MSIT) (NRF-2020R1A1C3004123).
Keywords: chondrogenesis, human induced pluripotent stem cell, IL-6

TOPIC: NEURAL

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USING SCN8A MUTANT HIPPOCAMPAL FUSION ORGANOIDS AS A MODEL TO STUDY PATHOGENESIS OF DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY-13 (DEE13)

Lozano, Kevin - *Neurology, University of California, Los Angeles, CA, USA*
Butlig, Evan-Angelo - *Neurology, University of California, Los Angeles, CA, USA*
Fotion, Namie - *Neurology, University of California, Los Angeles, CA, USA*
Kurdian, Arinnae - *Neurology, University of California, Los Angeles, CA, USA*
Samarasinghe, Ranmal - *Neurology, University of California, Los Angeles, CA, USA*

Abstract: Epilepsy is a neurological disorder most commonly characterized by sudden, recurrent seizures. Disease pathology has revealed a correlation to the abnormal development of neural networks. One region of particular interest is the hippocampus since hippocampal abnormalities are seen in multiple types of epilepsy. The hippocampus is located in the mesial region of the temporal lobe and plays a key role in the regulation, encoding, and consolidation of memory. This study focused on the gain of function mutation in the SCN8A gene since it is associated with a type of severe childhood epilepsy known as developmental epileptic encephalopathy-13 (DEE-13). The study of neural development has traditionally been performed using animal models, typically rodents, however, these models fall short when it comes to encapsulating the complexity of human brain development. Human brain organoids show promise in changing our understanding by better recapitulating normal and abnormal early embryonic development to study pathogenesis. Here, we generated hippocampal (Hc) and ganglionic eminence (GE) organoids from a patient derived human induced pluripotent stem cell line containing the SCN8A mutation and a CRISPR-Cas9 corrected isogenic control. We fused Hc and GE organoids in order to resemble the proper mix of inhibitory and excitatory neurons observed in vivo. Mut and iCtrl fusions were then compared between each other in order to assess differences in cell expression and subjected to local field potential (LFPs) in order to examine electrophysiology. Immunohistochemistry (IHC) experiments revealed that the mutation causes significant changes to the expression of cells affecting important structures of the Hc such as dentate gyrus and cornu ammonis, leading to the effects observed in DEE13. LFP experiments also revealed a difference between the iCtrl and Mut fusion organoids in the absence of important features such as sharp wave ripples in mutants.

Keywords: Epilepsy, Organoids, Stem cells

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UNRAVELING AXONAL DEGENERATION IN SPG3A USING HUMAN PLURIPOTENT STEM CELLS

Dhanukate, Rutuja Dashrath - *Department of Biomedical Sciences, University of Illinois at Chicago College of Medicine Rockford, IL, USA*
Li, Xue-Jun - *Department of Biomedical Sciences, University of Illinois College of Medicine Rockford, IL, USA*
Mou, Yongchao - *Department of Biomedical Sciences, University of Illinois College of Medicine Rockford, IL, USA*

Abstract: Hereditary Spastic Paraplegias (HSPs) are a group of inherited disorders characterized by axonal degeneration of corticospinal motor neurons, leading to progressive lower limb spasticity and gait disturbances. Spastic Paraplegia 3A (SPG3A), the most common early-onset form of HSP is caused by mutations in the ATL1 gene encoding for the protein Atlastin-1. No treatment options are currently available to prevent or reverse SPG3A. Here, we aim to develop a patient-specific human stem cell-based model to test protective effects of small molecules by reprogramming fibroblasts to induced pluripotent stem cells (iPSCs) and further differentiating them into cortical neurons. First, patient-specific fibroblasts were reprogrammed into iPSCs by using the sendai virus integration-free method. DNA sequencing confirmed the maintenance of patient-specific mutation in iPSCs. The qPCR data revealed that patient-specific iPSCs had higher expression of pluripotency genes namely OCT4, NANOG and SOX2 with relatively lower expression of fibroblast-specific FGF5, FGF8 and FGF9 genes. Fluorescent images obtained from immunocytochemistry staining confirmed that iPSCs uniformly expressed pluripotent proteins like SOX-2, SSEA-4 and NANOG. HE staining of teratoma sections from SCID mice revealed varying percentages of mesoderm, endoderm and ectoderm, confirming the pluripotency of iPSCs. SPG3A iPSCs were further differentiated into cortical projection neurons by our well-established method and plated to perform immunocytochemistry against TAU protein. The TAU immunostaining showed that SPG3A neurons had a significant reduction in the axonal outgrowth as compared to that in the control neurons. Furthermore, immunocytochemistry of cortical neurons against TAU and pNFH proteins was performed to quantify the axonal swellings and a small molecule compound demonstrated several fold reduction in axonal swellings when compared to the control. Notably, this compound is also capable of reducing apoptosis of SPG3A neurons determined by Caspase3/7 luminescent assay. In summary, our results demonstrate the recapitulation of disease-specific phenotypes in a human stem cell-based SPG3A model, thereby providing a unique system for identifying therapeutic agents for HSPs.

Funding Source: This study is supported by NIH, Carter Foundation for Neurologic Research and University of Illinois College of Medicine Rockford.
Keywords: iPSCs, Axonal degeneration, Human cortical neurons

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PROPERTIES OF HUMAN STEM CELL-DERIVED NEURONS IN 2D AND 3D IN LONG-TERM CELL CULTURE

Salmanzadeh, Hamed - *Physiology and Pharmacology, University of the Pacific, Stockton, CA, USA*
Halliwell, Robert F. - *Physiology and Pharmacology, University of the Pacific, Stockton, CA, USA*

Abstract: Studies in our lab are addressing the functional properties of receptors and ion channels expressed in neurons derived from human stem cells. In the current work, we utilized phase-contrast microscopy, immunocytochemistry and a multi-electrode array (MEA) system to determine the electrophysiological and pharmacological properties of human (TERA2.cl.SP12) stem cell-derived neurons in 2D and 3D in long-term (≤1 year) cell culture. The proportion of human stem cells differentiating in 2D to a neural phenotype, as indicated by β-III tubulin and MAP2 immunocytochemistry, increased over 3 months from approximately 5% at day 10 to ≥38% at day 80 in vitro. Glial cells, identified by GFAP labeling, first appeared at day 50 in vitro. Recordings from populations of stem cell-derived neurons showed spontaneous spike-like activity that was inhibited by several ion channel modulators. Spike rate, spike amplitude and synchronous firing all increased over time in culture and indicated neural network formation. Pharmacological experiments also indicated that neurotransmission in these neural networks was mediated via glutamate and GABAA receptors, the major excitatory and inhibitory systems in the brain, respectively. We also determined the functional properties of these stem cell-derived neurons and glia as 3D neurospheres (neural organoids), representing a more complex physiological model of the human nervous system in vitro. Cells within the organoids labeled with β-III tubulin and GFAP. We observed increased spontaneous spike-like activity, burst firing, and synchronized firing with increasing maturation in cell culture. Neurotransmission was largely mediated via glutamate and GABAA receptors. Neural networks also demonstrated epileptiform firing in response to convulsants which was inhibited using widely used anti-seizure drugs. Our results demonstrate the feasibility of utilizing stem cell-derived neurons in long-term culture and MEA measurements in vitro for functional assessments and drug screening.

Keywords: Stem cell-derived neurons, brain organoids, drug discovery model

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NEURODEVELOPMENTAL CONSEQUENCES OF RARE SETD1A MISSENSE VARIANTS ASSOCIATED WITH RISK FOR BIPOLAR DISORDER

Ament, Seth A. - *Department of Psychiatry and Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA*
Lease, Robert - *Program in Molecular Medicine, University of Maryland School of Medicine, Baltimore, MD, USA*
Oshone, Rediet - *Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA*
Cortes-Gutierrez, Marcia - *Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA*
Humphries, Elizabeth - *Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA*
Choe, Jayme - *Institute for Genome Sciences, University of*

Maryland School of Medicine, Baltimore, MD, USA
Ali, Samaneh - *Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA*
Detera-Wadleigh, Sevilla - *Human Genetics Branch, Intramural Research Program, National Institute of Mental Health, Bethesda, MD, USA*
Kochunov, Peter - *Maryland Psychiatric Research Center, University of Maryland School of Medicine, Catonsville, MD, USA*
Shuldiner, Alan - *Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA*
Mitchell, Braxton - *Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA*
Colantuoni, Carlo - *Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA*
McMahon, Francis - *Human Genetics Branch, Intramural Research Program, National Institute of Mental Health, Bethesda, MD, USA*
Hong, Elliot - *Maryland Psychiatric Research Center, University of Maryland School of Medicine, Catonsville, MD, USA*

Abstract: Rare variants with large effects provide excellent opportunities to characterize causal mechanisms for complex disorders. In recent large-scale exome sequencing studies, SET Domain Containing 1A (SETD1A), a chromatin remodeling gene, has emerged as the top, strongly supported risk gene for schizophrenia (P = 2.0e-12) and related disorders. To date, despite the prominence of SETD1A in neuropsychiatric risk, there have been no published studies of human neural cells with naturally occurring SETD1A variants, and very little is known about the effects of these variants on brain development or brain function. Here, we describe the discovery and characterization of rare SETD1A missense variants that are enriched in the Old Order Amish founder population. Individuals carrying Amish-enriched missense variants in SETD1A exhibited mild cognitive deficits and psychotic forms of bipolar disorder. In patient-derived induced pluripotent stem cells (iPSCs) and iPSC-derived neural lineages we found robust molecular and cellular phenotypes, including increased vulnerability to DNA damage, decreased cellular proliferation, inefficient formation of neural rosettes, reduced neurite outgrowth, and transcriptional and epigenomic signatures of premature cell cycle exit and neural differentiation. We demonstrate that a subset of these phenotypes can be rescued by pharmacological inhibition of the H3K4-specific demethylase KDM5. These results expand the SETD1A clinical phenotype and demonstrate deficits in neuronal development that may underlie risk for neuropsychiatric disease.

Funding Source: This study was supported by grants and contracts from the Maryland Stem Cell Research Fund, National Institute of Mental Health (U01 MH108148), Regeneron Genetics Center, and the NIMH Intramural Research Program (ZIA MH002843).
Keywords: schizophrenia, bipolar disorder, neurodevelopment

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MODELING THE INDUCTION OF REACTIVITY IN HUMAN PLURIPOTENT STEM CELL-DERIVED ASTROCYTES AND THEIR CONTRIBUTIONS TO NEURODEGENERATION

Meyer, Jason S. - *Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA*
Gomes, Cátia - *Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA*
Harkin, Jade - *Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, USA*
Huang, Kang-Chieh - *Biology, Indiana University Purdue University Indianapolis, IN, USA*
Lavekar, Sailee - *Biology, Indiana University Purdue University Indianapolis, IN, USA*

Abstract: Astrocytes are the most abundant cell type in the brain, where they closely associate with neurons to provide support via multiple mechanisms, but can also contribute to their neurodegeneration in a variety of disease states. However, the mechanisms by which astrocytes promote neurotoxicity and contribute to neurodegeneration remain unclear. Human pluripotent stem cells (hPSCs) can serve as powerful tools for the in vitro analysis of human neurodegenerative diseases, including neuron-glia interactions. Using hPSC-derived astrocytes and neurons, we explored how induced reactive astrocytes contribute to neurodegeneration. The induction of a reactive astrocyte phenotype was promoted through incubation with a cocktail of recombinant proteins including C1q, TNFα and IL1α. Reactive astrocytes displayed profound morphological alterations exhibiting a hypertrophic profile and increased expression of A1-reactive specific markers such as complement C3. Moreover, transcriptional analyses revealed an upregulation of genes associated with the inflammatory pathway as well as cytokine signaling in reactive astrocytes. Additionally, the secretion of several pro-inflammatory cytokines was increased in reactive astrocytes. Subsequently, the neurotoxic potential of reactive astrocytes was determined through co-cultures with a variety of hPSC-derived neurons, including retinal ganglion cells, cortical neurons, and spinal motor neurons, in which reactive astrocytes promoted marked morphological alterations including neurite retraction and reduced neurite complexity. Furthermore, the ability to more effectively model disease states in astrocytes following induction of reactivity was determined with cell lines from a variety of disease states. Overall, these results demonstrated that hPSC-derived astrocytes can be induced to acquire a reactive profile with a predominant inflammatory and neurotoxic phenotype which profoundly contributes to neurodegeneration. Thus, the modulation of reactive astrocytes could be a novel therapeutic strategy for neurodegenerative diseases.

Funding Source: R01EY033022, RF1AG069425
Keywords: astrocyte, reactivity, neurodegeneration

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MODELING C9ORF72 FTD/ALS USING A NEW ORGAN-CHIP MODEL TO INFORM CLINICAL TRIALS

Ondatje, Briana N. - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Lall, Deepti - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Artz, Madelyn - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Lukasiak, Michael - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Bell, Shaughn - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Lawless, George - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Seeley, William - *Department of Neurology, University of California, San Francisco, CA, USA*
Van Eyk, Jennifer - *Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Svendsen, Clive - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Frontotemporal dementia (FTD) is a common dementia syndrome in patients under age 65, and amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with the loss of spinal cord and cortical motor neurons leading to paralysis and death from respiratory failure typically within 3-5 years of diagnosis. FTD and ALS have significant overlap clinically, pathologically and genetically. Aggregates of TDP-43 protein are the defining pathology of FTD (FTLD-TDP variant) and nearly all cases of ALS, and the most common genetic cause of both FTD and ALS are repeat expansions in the C9orf72 gene. C9orf72 is expressed in multiple cell types in the brain including microglia and neurons, and there is strong evidence that disrupted interactions between different cell types underlie pathogenesis in C9-FTD/ALS. In order to model C9-FTD/ALS forebrain, we have developed a microphysiologic system (MPS) that permits the 3D culture of human induced pluripotent stem cell (iPSC)-derived cortical neurons (CNs), astrocytes and microglia in one chamber and brain microvascular endothelial cells in a separate chamber, separated by a porous membrane that provides a blood brain barrier component. Using C9-FTD/ALS patient-derived iPSCs, our goal is to validate the robustness of this forebrain MPS (fMPS) model and to identify C9-FTD/ALS-specific biomarkers, which we will then cross validate with the patient clinical data and pathology. In the future, the established fMPS model can be used to discover and validate translatable biomarkers for preclinical efficacy testing, and to assist in patient stratification for clinical trial design.

Funding Source: NIH grant #1UG3TR003264-01
Keywords: forebrain organ-on-chip, neurodegeneration, clinical trial

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MICROGLIA-SPECIFIC AD RISK VARIANT BIN1 DELETION IN IPSC-DERIVED MICROGLIA

Lopez Sanchez, Mark - *Biology, California State University, San Marcos, Homeland, CA, USA*

Abstract: Microglia, the primary immune cell type of the brain, plays a critical role in the immune response of neurodegenerative disorders such as Alzheimer's disease (AD). Although the pathogenesis of AD remains unknown, AD genetic risk variants have been found in a microglia-specific Bridging Integrator 1 (BIN1) gene locus, suggesting that dysregulation of microglial BIN1 contributes to AD risk. In addition, the expression of BIN1 is present in all cell types of the CNS, yet the function of BIN1 in Microglia remains unknown. To investigate the role of BIN1, we leveraged hESC and iPSC-derived microglia using CRISPR /Cas9 technology to create a BIN1 traditional loss of function (tKO) line. The tKO line CRISPR edit was done through a frameshift mutation in BIN1 exon 8 causing a loss of function. To address any functional changes caused by the deletion of BIN1 in Microglia, we utilized phagocytosis, cell migration assays, and mitochondrial function assays. In addition, lysotracker was used as a marker for lysosomal activity and Cellrox to measure oxidative stress. These different in-vitro functional assays were conducted with stimulation of amyloid- β and Tau to give an insight of any potential phenotypic changes. This will provide an understanding if the loss of BIN1 alters microglia function and potentially indicate a mechanism for how BIN1 enhances AD risk.

Keywords: microglia, neuro, Alzheimer's Disease

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METHADONE DISRUPTS DEVELOPMENTAL SYNAPSE FORMATION IN HUMAN CORTICAL ORGANOIDS

Dwivedi, Ila - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Dan, Zhou - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Caldwell, Andrew - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Subramaniam, Shankar - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Haddad, Gabriel - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*

Abstract: Over the past two decades, opioid use disorder (OUD) among pregnant women has become an epidemic in the United States. Clinical interventions for OUD involve methadone, a synthetic opioid analgesic that attenuates withdrawal symptoms and behaviors linked with drug abuse. However, methadone use during pregnancy has raised concerns regarding its effect on the fetal brain. This has been compounded by evidence of its increased accumulation in and reduced elimination from embryonic neural tissue, as well as its association with long-term cognitive sequelae. Our lab used iPSC-derived human cortical organoids (hCOs) to probe the effect of methadone on cellular and molecular brain development in vitro. We demonstrated that exposure to clinically relevant (1-10μM) doses of methadone suppress synaptic transmission in 2-3-month-old hCOs undergoing synapse formation. To investigate how methadone affects synaptogenesis, we conducted bulk mRNA sequencing of 2-month-old hCOs chronically treated with 1μM methadone for 50 days. Our analyses revealed a robust transcriptional response to methadone, with 2139 significantly differentially expressed genes (SDEGs, $|FC| \geq 1.5$, p-adj < 0.05). Preliminary ontology analyses indicated that these SDEGs were primarily enriched for cellular signatures associated with the synapse. In depth investigations into the molecular functions of these synapse associated genes have now revealed their involvement in pre- and post-synaptic specification mechanisms, such as vesicular trafficking, signal reception, and signal transduction. We also observed changes in the expression of several regulatory extracellular matrix (ECM) genes, including a key class of astrocyte-secreted matricellular proteins called thrombospondins that are known to modulate developmental synapse formation and a large number of their postulated interacting partners. Together, our results indicate that methadone's disruption of this ECM regulatory axis alters the recruitment and/or structural assembly of functional components during synaptogenesis in the fetal cortex. We believe these findings will provide novel insight into the mechanisms underlying adverse neurocognitive outcomes linked with prenatal opioid exposure and will elucidate potential new avenues for interventions in OUD.

Funding Source: National Institutes of Health (NIH) - National Institute on Drug Abuse (NIDA), UCSD School of Medicine - Department of Pediatrics & Respiratory Medicine, UCSD Jacob's School of Engineering, Rady Children's Hospital
Keywords: Neurodevelopment, Synaptogenesis, Opioids

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INVESTIGATING CELL TYPE-SPECIFIC VULNERABILITY IN PARKINSON'S DISEASE USING AN IN VITRO GENETIC MODEL

Sarrafha, Lily - *Department of Cell, Developmental, and Regenerative Biology, Nash Family Department of Neuroscience, Black Family Stem Cell Institute, Ronald M. Loeb Center for Alzheimer's Disease, The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Ahfeldt, Tim - *Department of Cell, Developmental, and Regenerative Biology, Nash Family Department of Neuroscience, Black Family Stem Cell Institute, Ronald M. Loeb Center for Alzheimer's Disease, The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Benson, Deanna - *Nash Family Department of Neuroscience, Ronald M. Loeb Center for Alzheimer's Disease, The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Blanchard, Joel - *Department of Cell, Developmental, and Regenerative Biology, Nash Family Department of Neuroscience, Black Family Stem Cell Institute, Ronald M. Loeb Center for Alzheimer's Disease, The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Sahasrabudhe, Abhishek - *Nash Family Department of Neuroscience, Ronald M. Loeb Center for Alzheimer's Disease and The Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract: Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic neurons (DNs) in the substantia nigra of the midbrain. PRKN (Parkin) is a familial PD gene which encodes a protein that mediates mitophagy and leads to mitochondrial dysfunction in DNs upon its loss of function. While midbrain DNs degenerate in PD, DN subtypes in the hypothalamus are relatively spared. However, the mechanisms of this region-specific vulnerability are largely unclear. To investigate the mechanisms underlying DN region-specific vulnerability, we developed tyrosine hydroxylase (TH) reporter hPSC lines that enabled us to optimize differentiation protocols for midbrain and hypothalamic organoids. We then generated isogenic Parkin-/- lines from the WT hPSC reporters and analyzed the emerging DNs in all groups upon differentiation. Similar to clinical studies, we have observed that Parkin-/- DNs in midbrain organoids exhibit increased cellular death whereas the hypothalamic Parkin-/- DNs do not. High-throughput imaging was used to compare mitochondrial phenotypes and revealed that anterograde mitochondrial transport is dysregulated in Parkin/- DNs in midbrain but not hypothalamic organoids, suggesting that mitochondrial dysfunction may have a central role in region-specific DN death. To identify the underlying mechanisms, we performed RNA sequencing which revealed that several mitochondrial proteins are selectively dysregulated in midbrain Parkin-/- organoids. We are currently employing chemical and genetic approaches to validate dysregulated pathways in our disease models. Collectively, results from this study will elucidate the molecular pathways that are differentially dysregulated in midbrain and hypothalamic DNs and help in identifying the cell-autonomous causes of vulnerability in PD, leading to novel therapeutic strategies.

Keywords: Neurodegenerative disorders, Organoids, Mitochondrial phenotypes

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EXPLORING GLIA-TO-NEURON REPROGRAMMING IN VIVO USING PRE-CLINICAL HUMANIZED RODENT MODELS

Habekost, Mette - *Department of Experimental Medical Science, Lund University, Lund, Sweden*
Giacomoni, Jessica - *Department of Experimental Medical Science, Lund University, Lund, Sweden*
Hoban, Deidre - *Department of Experimental Medical Science, Lund University, Lund, Sweden*
Goldman, Steven - *Department of Neurology and Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY, USA*
Parmar, Malin - *Department of Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder, which is associated with focal loss of dopaminergic neurons in the substantia nigra. The therapeutic prospective of cell replacement therapy for treating PD has been established through clinical trials transplanting human fetal tissue and stem cell therapies are under development. Direct reprogramming of resident glia cells in situ into therapeutic neurons represents a strategy based on cell replacement but circumvents the need for cell transplantation. Proof-of-concept experiments has been performed in rodent models using genetic overexpression of lineage-specifying transcription factors or downregulation of key barriers such as Ptbp1, but it remains an open question if human glia can be converted in situ into clinically relevant neurons with the ability to restore function in the damaged brain. For studies of direct neural reprogramming of human glia, we have established a stem cell-based in vitro conversion model (Nolbrant et al., 2020) as well as pre-clinical humanized rodent models for in vivo conversion. In the rodent models, glia progenitor cells (GPCs) derived from human embryonic stem cells are transplanted into adult or neonatal immunodeficient rats. The GPCs engraft and proliferate, and upon maturation the rodent brain contains large numbers of human GPCs and astrocytes. We have engineered the human cells to express CRE recombinase, which allows us to specifically target the human cells in vivo upon stereotactic injection of CRE-dependent neural conversion factors. Using these models, we are currently exploring both transcription factor-mediated conversion and PTBP1 knockdown-mediated conversion in vitro and in vivo, with the goal to select neural conversion factors that specify the reprogramming of dopaminergic neurons from human glia directly in the brain.

Funding Source: Swedish Research Council, Knut och Alice Wallenberg Foundation, Danmarks Frie Forskningsfond & Lundbeckfonden
Keywords: Direct Neural Reprogramming, Glia Progenitor Cells, Pre-clinical Humanized Rodent Models

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ESTABLISHING HPSC-DERIVED ENTERIC GANGLIOIDS TO MODEL HUMAN ENS DEVELOPMENT AND FUNCTION

Majd, Homa - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*
Samuel, Ryan - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*
Ramirez, Jonathan - *Cellular and Molecular Pharmacology,*

University of California, CA, USA
Kalantari, Ali - *Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA*
Barber, Kevin - *Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA*
Ghazizadeh, Zaniar - *Division of Cardiovascular Medicine, Stanford University, Stanford, CA, USA*
Chemel, Angeline - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*
Cesiulis, Andrius - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*
Richter, Mikayla - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*
Das, Subhamoy - *Department of Neurosurgery, Stanford University, Stanford, CA, USA*
Keefe, Matthew - *Department of Anatomy, University of California, San Francisco, CA, USA*
Wang, Jeffrey - *Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Shiv, Rahul - *Department of Electrical engineering, Stanford University, Stanford, CA, USA*
McCann, Conor - *Stem Cells and Regenerative Medicine, University College, London, UK*
Bhat, Samyukta - *Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA*
Khoroshkin, Matvei - *Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Yu, Johnny - *Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Nowakowski, Tomasz - *Department of Anatomy, University of California, sSan Francisco, CA, USA*
Goodarzi, Hani - *Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Thapar, Nikhil - *Woolworths Centre for Child Nutrition Research, Queensland University of Technology, Brisbane, Australia*
Kaltschmidt, Julia - *Department of Neurosurgery, Stanford University, Stanford, CA, USA*
Fattahi, Faranak - *Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA*

Abstract: The enteric nervous system (ENS) plays a critical role in gut physiology and the cross-talk between the gastrointestinal tract and other organs. The human ENS has remained elusive, emphasizing the need for an in vitro modeling and mapping blueprint. Here we establish human pluripotent stem cell (hPSC) differentiation strategies to generate robust and reliable 2D ENS cultures and 3D enteric ganglioids to map out the developmental and functional features of the human ENS. In addition to recapitulating the remarkable neuronal and glial diversity found in primary tissue, these models enable comprehensive molecular analyses that unravel functional and developmental relationships within these lineages. An example of the power and versatility of this system is our detailed characterization of enteric nitrergic neurons (NO) that are implicated in a wide variety of gut motility disorders. By performing unbiased high-throughput screening, we identified drug candidates that modulate the NO neuron activity and demonstrated their potential in promoting mouse colonic motility ex vivo. To define the developmental programs involved in NO neuron specification, we performed unbiased screening and discovered PDGFR inhibition boosts the induction of NO neurons in enteric ganglioids. When transplanted, these ganglioids engraft extensively into the colon of NO-neuron deficient mice, providing a xenograft model for the study of human ENS in vivo. Our studies offer a framework for understanding the fundamental characteristics of the human ENS and designing effective strategies to develop drugs and cell-based approaches to treat enteric neuropathies.

Funding Source: F.F. is supported by the NIH Director's New Innovator Award (DP2NS116769) and the NIDDK (R01DK121169). H.M. is supported by Larry L. Hillblom Foundation postdoctoral fellowship.
Keywords: enteric nervous system, nitrergic neuron, disease modeling

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EPIGENETIC LANDSCAPE AND TRANSCRIPTOME IN HUMAN NEURAL PROGENITORS AFTER IRRADIATION/LITHIUM EXPOSURE: IMPLICATIONS FOR TREATMENT OF COGNITIVE DEFECTS AFTER BRAIN RADIOTHERAPY

Neofytou, Christina - *Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden*
Backlund, Alexandra - *Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden*
Railaite, Vaida - *Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden*
Blomgren, Klas - *Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden*
Hermanson, Ola - *Department of Neuroscience, Karolinska Insitutet, Stockholm, Sweden*

Abstract: Cranial radiotherapy is commonly used to treat childhood brain cancers, but leads to late-effects, such as decline in cognition, mood and social competence, likely due to neurogenesis impairment. Only in Sweden, there are 11,000 pediatric cancer survivors (almost half as many as Parkinson's Disease patients), and 60-90% of them suffer from late-effects. Yet, little effort has been made to improve their life quality. Lithium exerts neuroprotective, pro-neurogenic, and antitumor effects, and it reverses radiation-induced damage in rodents, through increased levels of GAD2. In this study, lithium treatment of irradiated iPSC-derived human neural stem and progenitor cells (hNSPCs) lead to GAD2 upregulation, followed by decreased promoter methylation levels [Δβ(IR+LiCl-Cntl)=-0.04346], as shown in gene expression and methylation arrays, respectively. We also observed a five-fold increase in GREM1 (Gremlin, a BMP inhibitor) in irradiated and lithium treated hNSPCs compared to control, while its promoter region was hypomethylated [Δβ(IR+LiCl-Cntl)=-0.1301]. We propose that lithium may promote neuronal differentiation by regulating GAD2 expression and can inhibit the glial switch through GREM1-mediated BMP signaling repression. The mechanism of the methylation changes is still unknown. We revealed a two-fold increase in GADD45A gene expression, associated with decreased DNA methylation of its promoter [Δβ(IR+LiCl-Cntl)=-0.028033], as well as a significant increase in TET3 expression. GADD45A may recruit and activate TET3; such a model could explain the selective regulation of DNA demethylation on individual genes after lithium treatment in irradiated hNSPCs. Current experiments aim at revealing the genome-wide changes in DNA methylation and associated gene expression after lithium treatment in irradiated cells, with focus on the role of TET3. For this, we employed siRNA-mediated reduction of TET3, catalytic domain inhibition with the use of TETIN-C35, and increased activity of TETs after Vitamin C treatment. Taken together, our observations strengthen the potential for pharmacological treatment of cognitive late-effects in childhood cancer survivors with the use of lithium and suggest

that TET3-mediated demethylation may be a key mechanism of action.

Funding Source: The Swedish Childhood Cancer Foundation, The Swedish Cancer Society, Cancer Research KI.
Keywords: Epigenetics, Brain tumor, Late effects

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MODELLING NEURODEGENERATION USING A HUMAN ISOGENIC SYSTEM: A NEXT GENERATION APPROACH TO STUDY HUNTINGTON'S DISEASE

Roisin, Nicoll - *Product Development, bit.bio, Cambridge, UK*
Abstract: The development of therapies to treat patients with neurodegeneration is hampered by the use of animal models, less than 10% of findings derived from these pre-clinical models translate to humans. Patient-derived induced pluripotent stem cells (iPSCs) can generate in vitro systems to model neurological diseases that recapitulate relevant human disease phenotypes. Conventional human iPSC (hiPSCs) differentiation protocols are lengthy, inconsistent, and difficult to scale. There is a lack of isogenic controls lines for iPSC-derived disease models, complicating studies on disease relevant phenotypes and molecular mechanisms as well as drug discovery. To overcome these problems, we developed a proprietary gene-targeting strategy (opti-ox) that enables highly controlled expression of transcription factors to rapidly reprogram hiPSCs into pure somatic cell types in a scalable manner. CRISPR/Cas9 gene editing allows the introduction of specific mutations in iPSC-lines and creates isogenic disease models that improve screen specificity and accelerate drug development. We have developed opti-ox based isogenic ioGlutamatergic Neurons Huntington's disease (HD) model carrying a 50CAG expansion in the HTT gene. Mutant HTT proteins containing elongated polyglutamine stretches are aggregation-prone and affect a range of neuronal subtypes, including cortical glutamatergic neurons. Characterisation of these neurons by ICC and RT-qPCR showed the expression profile of pan-neuronal (MAP2 and TUBB3) and glutamatergic (VGLUT1 and VGLUT2) marker genes as well as of the HTT transcript itself are highly similar between ioGlutamatergic Neurons HTT 50CAG and the isogenic control. We are currently performing an in-depth phenotypic characterisation of this HD disease model and the wildtype isogenic control to determine the differences in their transcriptome, neuronal activity and mitochondrial functions. Beside the 50CAG mutation in HTT, we have generated mutations in MAPT, TARDBP, GBA and PARKIN to provide isogenic disease models for FTD, FTD/ALS and Parkinson's disease. Our novel strategy to use the opti-ox technology for the scalable and consistent production of iPSC-derived isogenic disease models, offers new avenues into drug discovery and can accelerate research and the development of new therapeutics.

Funding Source: N/A
Keywords: Huntington's Disease, Disease Modelling, Neurodegeneration

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DEVELOPMENT OF TAU MISEXPRESSION SYSTEMS IN BRAIN ORGANOIDS FOR MODELING AD PROGRESSION AND THERAPEUTIC DISCOVERY

Heinrichs, Eric - *Neurobiology, University of California, Los Angeles, CA, USA*
Seidler, Paul - *Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA, USA*
Eisenberg, David - *Chemistry and Biochemistry, University of California, Los Angeles, CA, USA*
Novitch, Bennett - *Neurobiology, University of California, Los Angeles, CA, USA*

Abstract: Alzheimer's Disease (AD) affects approximately 5.8 million Americans and costs over $500 billion in care costs and informal care opportunity costs each year. Despite decades of research studying this disease, there are currently no effective cures or treatments that can do more than temporarily slow the disease. We believe that one of the most likely reasons extensive study has not yet yielded successful treatments is that the model systems being used are missing crucial pieces. Much of the work conducted thus far has been based on two-dimensional cell culture systems or mice engineered to exhibit features of the disease which do not normally occur in nature. These systems also have limitations in their ability to accurately model the structural and functional complexity of the human brain. Moreover, many studies have primarily focused on amyloid beta as the neurotoxic component of AD, while more recent research suggests that tau, a protein pathologically indicated in a variety of dementias, plays a crucial role in this process. Here, we will present new approaches for modeling the effects of tau in AD using pluripotent stem cell-derived brain organoids. To induce tau pathology, we have developed means to overexpress different tau forms in organoids. These methods result in foci of tau hyperphosphorylation which is an early step in the progression of AD and other tauopathies. Extraction of the sarkosyl insoluble fraction from homogenates of mutant tau expressing organoids yields fibril structures visible under electron microscopy, further recapitulating the in vivo pathology. Homogenates of these mutant tau expressing organoids are also capable of seeding aggregation in tau biosensor cells. We will present our progress towards further defining this model system and using it as a means for investigating the pathogenesis of AD in human brain tissue. Ultimately we plan on using this system to test the efficacy of new tau aggregation blockers to halt disease progression.

Funding Source: This project was supported by funding to B.G.N. and D.E. from the UCLA Broad Stem Cell Research Center, the UCLA Steffy Brain Aging Fund, NICHD (P50HD103557), NIDA (R01DA051897), and the NIA (R56AG070895).
Keywords: Organoid, Alzheimer's, Tauopathy

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CHARACTERIZING THE HETEROGENEITY OF CENTRAL NERVOUS SYSTEM MACROPHAGES USING A NOVEL HUMAN-SPECIFIC ASSEMBLY OF HEMATOPOIETIC PROGENITORS WITH CEREBRAL ORGANOIDS ON A DE NOVO VASCULATURE PLATFORM

Yarmand, Amin - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*
Jian, Tiger - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*
Sivitilli, Adam - *Donnelly Center, University of Toronto, ON, Canada*
Attisano, Liliana - *Donnelly Center, University of Toronto, ON, Canada*
Pelletier, Laurence - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*
Wrana, Jeff - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*

Abstract: Recent advances in human pluripotent stem cell (hPSC)-derived brain organoids have shown great promise of uncovering unique aspects of human brain development. However, many studies have focused on generating individual components of the brain. To gain a better understanding of the brain development, various components must be assembled to recapitulate the complexity arising from the interplay between different cell types. The early brain development involves an assembly of a peri-neural vasculature plexus with neuronal and glial precursor cells concurrent to arrival of macrophage precursors generated via hematopoiesis events outside of the brain. Published studies have shown that hPSC-derived human hematopoietic progenitor cells (hHPCs) generate central nervous system (CNS) macrophages in the dish and upon xenotransplantation in mice. Yet, the utility of these cells in a de novo human specific model of vascularized brain remains un-characterized. Here, utilizing our previously established vascular in vitro organoid systems (VIVOS) platform, we have focused on the integration of hHPCs with human brain cerebral organoids (hCOs) and their development in a vascularized brain environment. hHPCs differentiate to CNS macrophage-like cells when transferred with hCOs onto VIVOS. hHPC-derived cells ramify and express markers of resident CNS macrophages on VIVOS. Comprised of vessels, astroglia, and neurons, VIVOS recapitulate the early microenvironment of CNS macrophage precursors and likely induce the CNS macrophage differentiation program in hHPCs. Furthermore, hHPC-derived CNS macrophages integrate with this environment and phagocytose neuronal and synaptic debris. To further interrogate the heterogeneity and complexity of our model, we plan to perform single cell transcriptomics under homeostatic and inflammatory conditions to allow for a functional study of CNS macrophage-like cells on VIVOS.

Funding Source: This research is part of University of Toronto's Medicine by Design initiative which receives funding from Canada First Research Excellence Fund (CFREF).
Keywords: Brain development, CNS macrophages, Vascularized brain

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AN EVOLUTIONARY MODEL FOR HUMAN-SPECIFIC VULNERABILITIES IN DOPAMINERGIC NEURONS

Nolbrant, Sara - *Department of Neurology, University of California, San Francisco, CA, USA*
Schaefer, Nathan - *Department of Neurology, University of California, San Francisco, CA, USA*
Wallace, Jenelle - *Department of Neurology, University of California, San Francisco, CA, USA*
Schmitz, Matthew - *Department of Neurology, University of California, San Francisco, CA, USA*
Pavlovic, Bryan - *Department of Neurology, University of California, San Francisco, CA, USA*
Pollen, Alex - *Department of Neurology, University of California, San Francisco, CA, USA*

Abstract: The human brain has evolved remarkable cognitive and social abilities over the last 6 million years, but these adaptations involved tradeoffs that may leave us more vulnerable to neurological disorders. Dopaminergic (DA) neurons of the ventral midbrain may be under particular stress in the human brain because their target regions in the striatum and cortex have disproportionately increased in size relative to the DA nuclei, and require increased functional innervation compared to other primates. We hypothesize that cell intrinsic gene regulatory changes related to cellular stress and metabolism evolved in human DA neurons in the context of this altered cellular ecology. To test this hypothesis, we developed interspecies ventral midbrain organoid models. We differentiated induced pluripotent stem cell-lines from human (n=8), chimpanzee (n=7), orangutan (n=1) and macaque (n=1) individuals together into ventral midbrain progenitors and matured these into DA neurons in a pooled 3D environment. These interspecies organoids develop numerous FOXA2/LMX1A/TH positive cells, indicative of a DA identity. To study divergence in gene expression and chromatin accessibility during DA neuron development, we performed single-nucleus RNA-seq and ATAC-seq analysis across stages of midbrain neuron specification (D16) and maturation (D40 and D100). Single cell gene expression analysis of these cultures revealed a large diversity of cell types, including identities corresponding to the full rostral-caudal extent of developing ventral midbrain. We observed homologous cell types between humans and chimpanzees, including DA progenitors and neurons, enabling the direct comparison of gene expression and accessible chromatin between species. In addition, to further unmask genetic variation in regulatory elements and gene expression related to vulnerabilities, mature DA organoids were exposed to rotenone to induce oxidative stress, which leads to selective loss of TH+ neurons. Collectively, this study helps illuminate the genomic mechanisms underlying human neuronal specializations and the selective vulnerability of DA neurons and could translate to a better understanding of disorders involving dysregulation or degeneration of DA neurons.

Funding Source: Alex Pollen is a New York Stem Cell Foundation Robertson Investigator.
Keywords: Dopaminergic neurons, Brain evolution, iPSCs

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3D SPINAL CORD ORGANOIDS FROM HUMAN AMNIOTIC FLUID-DERIVED PLURIPOTENT STEM CELLS: A NOVEL CELL SOURCE FOR DISEASE MODELING AND TREATMENT IN SPINA BIFIDA

Biancotti, Juan C. - *Surgery, Johns Hopkins University, Baltimore, MD, USA*
Sferra, Shelby - *Surgery, Johns Hopkins University, Baltimore, MD, USA*
Penikis, Annalise - *Surgery, Johns Hopkins University, Baltimore, MD, USA*
Miller, Jena - *Gynecology - Obstetrics, Johns Hopkins University, Baltimore, MD, USA*
Kunisaki, Shaun - *Surgery, Johns Hopkins University, Baltimore, MD, USA*

Abstract: Myelomeningocele (MMC) is a severe form of spina bifida resulting from failed fusion of the caudal region of the neural tube during embryonic development. Despite early prenatal diagnosis and neurosurgical closure of the defect in utero or shortly after birth, affected children still suffer substantial and permanent disabilities. Most of the research is conducted in small and large animals; however, the mechanism of disease in humans cannot be clearly determined using these models. The purpose of this study was to generate 3D dorsal spinal cord organoids from human MMC amniotic fluid-derived induced pluripotent stem cells (iPSCs). Two MMC and one control iPSCs were successfully generated from 22 weeks gestation amniotic fluid samples, as demonstrated by characteristic morphology, alkaline phosphatase staining, positive expression of pluripotency markers (e.g., NANOG, OCT4, SOX2, SSEA3), and formation of embryoid body expressing markers of the three germ layers. Differentiation into 3D spinal cord organoids following a modified Takahashi group protocol was shown by quantitative gene expression, demonstrating at day 15 neuroectoderm (SOX1, PAX6), roof plate (Lmx1a), dorsal (Olig3, PAX7), and ventral (Olig2) spinal cord progenitors. Following 9 days of dorsalization with BMP4 morphogen, day 24 organoids expressed Brn3a and Lbx1 markers of dorsal interneurons and Lhx3/Islet2 markers of motoneurons. The presence of oligodendrocyte progenitors and astrocytes was revealed by immunostaining with NG2 and GFAP antibodies, respectively. Caudalization with bFGF drives cells towards the thoracic/lumbar level. Overall, MMC cell lines had significantly fewer neuroectodermal cells than control line at day 9 of differentiation, and progenitors/neurons were more restricted to the dorsal spinal cord as observed at day 24. For the first time, this pilot study suggests that human 3D dorsal spinal cord organoids can be successfully generated from MMC amniotic fluid iPSCs in as little as 3 months. This novel approach may provide investigators with an in vitro platform to study MMC spinal cord development and disease pathogenesis. Additionally, dorsal spinal cord organoids may represent an important autologous source of neural progenitors for spinal cord regeneration at the time of fetal or neonatal MMC repair.

Funding Source: NIH - NICHD R01 HD091323
Keywords: Organoids, Spina bifida, Amniotic fluid

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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AN ITSC-DERIVED STEM CELL MODEL FOR SARS-COV-2 INFECTION OF EARLY PLACENTAL DEVELOPMENT REVEALS SUSCEPTIBILITY AND MECHANISMS FOR DRUG DEVELOPMENT AND THERAPIES

Chen, Joseph - *Australian Regenerative Medicine Institute, Monash University, Clayton, Australia*
Neil, Jessica - *Department of Microbiology and Immunology, The University of Melbourne, Australia*
Tan, Jia - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Rudraraju, Rajeev - *Department of Microbiology and Immunology, The University of Melbourne, Australia*
Mohenska, Monika - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Sun, Yu - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Sun, Guizhi - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Zhou, Yichen - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Li, Ying - *School of Health and Biomedical Sciences, RMIT University, Bundoora, Australia*
Drew, Damien - *Infectious Diseases and Immune Defences Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia*
Pymm, Phillip - *Infectious Diseases and Immune Defences Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia*
Tham, Wai-Hong - *Infectious Diseases and Immune Defences Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia*
Rossello, Fernando - *Australian Regenerative Medicine Institute, Monash University, Clayton, Australia*
Nie, Guiying - *School of Health and Biomedical Sciences, RMIT University, Bundoora, Australia*
Liu, Xiaodong - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Polo, Jose - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Subbarao, Kanta - *Department of Microbiology and Immunology, The University of Melbourne, Australia*

Abstract: Despite several clinical reports, the implications of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on the pathophysiology of early placentation and pregnancy are not fully clear. In part, this is due to the difficulty in evaluating and studying SARS-CoV-2 during the early stages of human development. To shed light on the capacity of SARS-CoV-2 to infect early placental cells and the possible molecular and cellular consequences, we utilised our recently developed induced trophoblast stem cells (iTSCs) to generate an in vitro model of early placenta. We identified the expression of two genes, ACE2 and TMPRSS2, that encode proteins that are critical for SARS-CoV-2 virus entry, are expressed in placenta-specific cell types including the extravillous trophoblasts (EVTs) and especially, syncytiotrophoblasts (STs). Interestingly, despite the expression of ACE2 and TMPRSS2 in both placental cell types, only STs were infected. Further, we demonstrated that infected STs lack the ex-

pression of key cellular structure and differentiation genes, which led to impairment of cellular processes and morphology that are vital for their function. Importantly, infected STs also produced significantly lower amounts of human chorionic gonadotropin, which is important for the proper maintenance of pregnancy. We also showed that anti-ACE2 antibody prevents SARS-CoV-2 infection and restored normal ST differentiation and function. Finally, we identified a cathepsin-mediated virus entry mechanism in STs through antiviral drug screens and found specific drugs that may help curb placental infections. In summary, we have established a platform to study early placental cell types and provided a scalable and tractable system for large scale drug screens for therapy. We highlight the use of this platform to study strategies to protect the placenta during early pregnancy and development.

Funding Source: MRFF grant (MRF9200007), MRFF grant (GNT2002073) and DHHS Victorian Government Grant.
Keywords: Trophoblast, In vitro model, SARS-CoV-2

TOPIC: PLURIPOTENT STEM CELLS

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SYSTEMATIC UTILIZATION OF HUMAN IPSC-DERIVED CELL TYPES FOR SARS-COV-2 INFECTION AND DRUG DISCOVERY

Jethmalani, Yogita - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Eastman, Richard - *Division of Pre-Clinical Innovation, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Tristan, Carlos - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Inman, Jason - *Informatics Core, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Voss, Ty - *Imaging Specialist, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Slamecka, Jaroslav - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Jovanovic, Vukasin - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Ryu, Seungmi - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Chu, Pei-Hsuan - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*
Singec, Ilyas - *Stem Cell Translation Laboratory, NIH - National Center for Advancing Translational Sciences, Rockville, MD, USA*

Abstract: The COVID-19 pandemic is responsible for the death of more than 5.8 million people worldwide and remains a global public health challenge. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus) which can lead to acute pulmonary symptoms, systemic inflammation (e.g., cytokine storm), and induce pathological changes in various other organ systems, some of which can become chronic and difficult to diagnose and treat. In this study, we focused on the systematic analysis of human cell types ranging from cardiomyocytes, choroid plexus cells, different types of neurons and trophoblast stem cells derived from induced pluripotent stem cells (iPSCs) to model and better understand SARS-CoV-2 infection. The identification of susceptible human cell types that express native surface receptors then enabled us to perform high-throughput screening and identify approved drugs that showed antiviral activity. We propose that the combined use of relevant human cell types generated at scale from iPSCs and drug repurposing may become a key strategy for developing new urgently needed treatments for SARS-CoV-2 infections.

Keywords: SARS-CoV-2, iPSC model, drug discovery

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PRO-CHONDROGENIC EFFECT OF DECELLULARIZED EXTRACELLULAR MATRIX SECRETED FROM INDUCED PLURIPOTENT STEM CELL-DERIVED CHONDROCYTES

Choi, Si Hwa - *College of Medicine The Catholic University of Korea, CiSTEM Lab, Seoul, Korea*
Ju, Ji Hyeun - *Internal Medicine, Catholic University of Korea, Seoul, Korea*

Abstract: Decellularized organs and tissues have generated much interest in functional bioscaffolds to mimic the natural ECM in tissue engineering. Due to the allogeneic and heterogeneous risk of pathogen transmission and host immune response, studies have recently been conducted on natural extracellular matrix (ECM) extracted from cells in vitro. ECM derived from a cell type has different characteristics depending on the cell type, and it has been found that such ECM preferentially directs differentiation into a cell-specific lineage when the tissue origin of the cell coincides with the ECM. Therefore, we expected that in cell culture in ECM extracted from cells, function was regulated through signal transduction between cells and ECM. To test this, we proceeded with chondrocyte differentiation using induced pluripotent stem cells generated by reprogramming of cord blood. The produced chondrocytes (iChondrocytes) were differentiated in gelatin-coated tissue culture polystyrene (TCP). Accordingly, the groups were divided into a tissue culture polystyrene plate (TCP), a gelatin-coated plate (GEL), and a plate obtained by decellularization of iChondrocytes (ECM). We report the characteristics and mechanisms of cell-ECM interaction through recellularization of iChondrocytes with these groups, suggesting their potential as a potential non-cellular resource for tissue engineering and future applications.

Keywords: hiPSC derived chondrocyte(iChondrocyte), Decellularization and recellularization, Non-cellular resource

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HIGH-EFFICIENCY GENERATION OF MUTANT ISOGENIC PLURIPOTENT STEM CELLS FOR DISEASE MODELING OF CONGENITAL DYSERYTHROPOIETIC ANEMIA

Nandy, Krittika - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Rani, Sonam - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
D. V., Phaneendra - *Department of Hematology, Christian Medical College, Vellore, India*
Kumarasamypet, Mohankumar - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Marepally, Srujan - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*

Velayudhan, Shaji - *Department of Hematology, Centre for Stem Cell Resaerch, Christian Medical College, Vellore, India*

Abstract: Congenital dyserythropoietic anemias (CDAs) are a highly heterogeneous set of rare anemias characterized by abnormal erythroid morphology and dyserythropoiesis. This disease is caused by mutations in CDAN1, C15ORF41, SEC23B, KLFI or KIF23 genes. CRISPR/Cas9 mediated double-stranded break followed by homology-directed repair (HDR) with an exogenous donor template has been used for creating missense mutations in iPSCs for isogenic disease modeling. However, this process is tedious, requiring clonal isolation and screening due to the extremely low rates of HDR. Base editing is a much simpler process that employs an adenine base editor (ABE) or a cytosine base editor (CBE) to convert A: T to G: C or C: G to T: A base pairs, respectively, within a specified activity window. As ABE results in more precise outcomes, we used this method to generate CDAN1 mutations to create an isogenic disease model for CDA Type I. The target mutations were chosen from a database of the reported disease-causing mutations and the gRNAs were designed by placing the base to be converted within the editing window. The gRNAs with high predicted efficiency were chosen to create F360L and T884A mutations in normal individual-derived iPSCs. We found that the ABE mRNA is highly efficient in generating a homogenous pool of gene-edited cells obviating the requirement of single-cell sorting and characterization. The mutant iPSCs produced comparable numbers of hematopoietic progenitor cells (HPCs) on cytokine-directed differentiation. However, the subsequent in vitro erythropoiesis recapitulated dyserythropoiesis observed in the CDA type I patients. Compared to the wild-type iPSCs, the HSPCs derived from the mutant iPSCs formed 10 times less erythroid colonies in the colony-forming assay, the mutant erythroid progenitors also exhibited significantly reduced proliferation and presented with maturation defects and inter-nuclear chromatin bridges characteristic of CDA. This study highlights a one-step inexpensive and efficient method to generate isogenic mutant iPSCs within five days without the requirement of extensive single-cell screening which takes several weeks to identify mutant iPSCs.

Funding Source: Depart of Biotechnology, Govt. of India and DBT-Welcome Alliance
Keywords: iPSC, Base Editing, Disease Modelling

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GENERATION, GENOMIC CHARACTERIZATION AND DIFFERENTIATION OF TRIPLOID HUMAN EMBRYONIC STEM CELLS

Haim, Guy - *Genetics, The Hebrew University, Jerusalem, Israel*
Golan-Lev, Tamar - *Genetics, The Hebrew University of Jerusalem, Israel*
Koren, Amnon - *Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA*
Benvenisty, Nissim - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Triploidy in human embryos is responsible for ~10% of all spontaneous miscarriages, and surprisingly, some of these pregnancies proceed to term. These triploid infants suffer from many physical and mental deficiencies, usually leading to their early death. To investigate the impact of triploidy on human development, we generated triploid human embryonic stem cells (hESCs) by fusing isogenic haploid and diploid hESCs. We show that triploid hESCs maintain typical morphology and pluripotency, but have a larger volume than the diploid cells, leading to decreased surface area to volume ratio. We analyzed the genome-wide transcription, methylation, and replication timing profiles of these cells and identified differentially expressed genes linked to several transport and metabolic processes. We analyzed the membranal proteins expression in triploid hESCs, identifying a downregulation of ion channels, resulting from the cells' altered dimensions. Interestingly, we observed an inhibition in in vivo differentiation of the triploid hESCs, especially to the neural lineages. This effect on neural cells may correspond to the downregulation of ion channel genes in triploid cells, as we demonstrate that many of these genes are more essential in neural progenitor cells than in undifferentiated cells. Notably, our research raises the possibility that triploidy-related pathologies may emerge due to their physical dimensions, affecting their initial developmental stages, and emphasizes our unique platform in studying the effects of ploidy on early human development.

Keywords: EARLY DEVELOPMENT, PLURIPOTENT STEM CELLS, TRIPLOIDY

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GENERATION AND ANALYSIS OF CEREBRAL ORGANOIDS FROM AUTISM PATIENTS WITH GERMLINE PTEN MUTATION AND MACROCEPHALY

Bogoniewski, Aleksander - *Neuroscience, University of California, Los Angeles, Valencia, CA, USA*

Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impairments in communication and social skills. Numerous genetic alterations are associated with ASD, including a subset of patients with macrocephaly that have mutations that activate the mTOR pathway. Prior studies in mice from our laboratory and others have shown that deletion of PTEN, an inhibitor of the mTOR pathway, results in enhanced neural stem cell proliferation and altered differentiation. Here we used cortical and subpallial cerebral organoids differentiated from patient derived iPSCs harboring the heterozygous H93Y PTEN mutation along with corresponding parental, and isogenic CRISPR corrected controls to study organoid growth and differentiation. We found that PTEN mutant organoids were only slightly larger than controls at day 50, suggesting a link between heterozygous PTEN deletion and macrocephaly.

Keywords: Organoid, Neuron, Autism

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CHANGES IN OXYGEN TENSION ON DEVELOPING VASCULARIZED LUNG

Loshbaugh, Chandler - *Biology, California State University, San Marcos, CA, USA*

Abstract: Human infants born prematurely have underdeveloped lungs that are less able to independently exchange gas. Premature infants have not developed sufficient pulmonary surfactant that can lead to respiratory distress syndrome (RDS). RDS is the leading cause of death in premature infants. In-utero, the lung develops in hypoxic conditions, so the premature transition to normoxia (ambient air) can be potentially injurious. However, it is not currently known how changes in oxygen levels influence the development of the human lung ex-utero. Therefore, we performed experiments to elucidate the role oxygen tension plays on vascularized lung epithelium development, and how oxygen

tension regulates cell differentiation and the expression of surfactant. First, we evaluated the protein expression of pulmonary surfactants (SPB and SPC) and SCGB3A2 in iPSC-derived lung and endothelial co-cultures representing the preterm lung by maturing them in hypoxic conditions and compared them to control cultures that matured in normoxia. We found hypoxic conditions induced greater differentiation of club and alveolar type 2 cell expressing proteins. We also showed normoxic and hypoxic conditions upregulated different isoforms of SPB. Further investigations will determine how hypoxia impacts the expression of HIF 3α, alveolar cells, club cells, surfactant proteins and lung development in a vascularized model for potential RDS therapies.

Keywords: Respiratory Distress Syndrome, Surfactant Protein, Lung

TOPIC: ETHICAL, LEGAL AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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RECAPITULATION OF FIRST PASS METABOLISM USING 3D PRINTED MICOFLUDIC CHIP AND ORGANOID

Lee, Kyung Jin - *Research and Development, ORGANOIDSCIENCES, Seongnam-si, Korea*
Kim, Do-Kyung - *Orthopaedic Surgery, CHA University, Seongnam, Korea*
Kim, Hyejin - *BD, ORGANOIDSCIENCES, Seongnam, Korea*
Jo, Suyoung - *BD, ORGANOIDSCIENCES, Seongnam, Korea*
Lee, Soonchul - *Orthopaedic Surgery, CHA University, Seongnam, Korea*
Yoo, Jongman - *CEO, ORGANOIDSCIENCES, Seongnam, Korea*

Abstract: The low bioavailability of oral drugs due to first pass metabolism is a major limitation in drug development. Recently, a three-dimensional (3D) printed microfluidic chip and 3D culture are getting attention as a potential solution in the development and evaluation of new drug candidates. The goal of this research was to create a microfluidic device that could mimic and analyze the first-pass metabolism. To visualize and preserve the organoid or spheroid on the chip, the infill condition of the polycarbonate transparent filament and layer height were optimized. Following that, a 3D printer was used to construct the chip after a computer-aided design (CAD). The chip was designed to have three wells and different height for gradient force. On the second and third wells, the SI organoid and colorectal adenocarcinoma spheroids, respectively, were placed. The tilted tunnel was connected to each well to carry the material by gradient force, and no extra equipment was required. Among three chip prototypes (chips 1, 2, and 3), Chip 2 had the highest distribution of plasmids in the Matrigel of the second well at 48h. Docetaxel was used to study the influence of first-pass metabolism. The viability of colorectal adenocarcinoma spheroids was significantly reduced in the chip without an SI organoid. However, there was no noticeable change in the chip with the SI organoid due to first pass metabolism. Together, we demonstrated a simple, quick, and low-cost microfluidic chip for analyzing the efficacy change of a candidate drug caused by first-pass metabolism.

Keywords: Organoids, organoid-on-a-chip; metabolism drug

TOPIC: EARLY EMBRYO

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IN VITRO MODEL OF HUMAN SOMITOGENESIS REVEALS COMPLEMENTARY ROLES FOR FGF AND WNT IN REGULATING SEGMENTATION CLOCK

Yaman, Yusuf Ilker - *Applied Physics, Harvard University, Allston, MA, USA*
Ramanathan, Sharad - *Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA*

Abstract: Somitogenesis is orchestrated by spatiotemporally structured gene expression dynamics and signaling gradients. However, it has been difficult to study the dynamics of human somitogenesis due to challenges working with human embryos. Using human pluripotent stem cells, we develop an in vitro human somitogenesis model, in which hundreds of organoids reproducibly recapitulate both traveling waves of gene expression and sequential somite segmentation. Through immunostaining and single-cell RNA sequencing, we show that these organoids faithfully reflect the anteroposterior organization and morphology of the human embryo by generating an elongating neural tube flanked by paraxial mesoderm, which further segments into somites. Diffusion map analysis of scRNA-seq data reveals the differentiation trajectory of neuromesodermal progenitors into somites and gradients of FGF and WNT ligands along the anteroposterior axis. Through live imaging of a dual-fluorescent reporter cell line tagging an oscillatory NOTCH target gene (HES7) and a somitogenesis marker gene (MESP2), we demonstrate that oscillating NOTCH activity sweeps through the presomitic mesoderm from the posterior to the anterior end and generates a new segmented somite for every oscillation. To understand how traveling waves are generated and modulated by FGF and WNT signaling gradients, we develop a mathematical framework that models the FGF, WNT and NOTCH pathways as coupled oscillators. Our model suggests a differential coupling mechanism for the FGF and WNT pathways along the anteroposterior axis, regulating NOTCH pathway oscillation dynamics. Altering FGF and WNT signaling through biochemical perturbations validates the complementary roles of FGF and WNT pathways in the generation of traveling waves of gene expression. Our study provides a reproducible model system to study human axial patterning, morphogenesis, and genetic musculoskeletal disorders, while proposing a novel mechanism to explain the generation of traveling waves of gene expression by coupling signaling gradients to the segmentation clock.

Keywords: Somitogenesis, Segmentation, Organoid

TOPIC: CARDIAC

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METABOLIC REGULATION OF CARDIAC MUSCLE CELL MATURATION

Nakano, Haruko - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Christofk, Heather - *Biological Chemistry, University of California, Los Angeles, CA, USA*
Ma, Feiyang - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*

Nakano, Atsushi - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Pellgrini, Matteo - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Kawahira, Naofumi - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Perez-Ramirez, Cesar - *Biological Chemistry, University of California, Los Angeles, CA, USA*
Thompson, Jeniffer - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*

Abstract: Metabolic environment is a critical determinant of cell fate and functions. In particular, hyperglycemia during pregnancy is associated with a 5-fold increase in the risk of congenital heart and neural tube malformations. We previously found that glucose inhibits the maturation of cardiomyocytes through the pentose phosphate pathway using the chemically-defined in vitro differentiation system of hPSC-derived cardiomyocytes (hPSC-CMs). Screening of the metabolites and various inhibitors of metabolic pathways revealed that elevated nucleotide abundance is inhibitory to cardiomyocyte maturation in a hyperglycemic environment. However, little is known about how high environmental glucose impacts in vivo developing organs. In this study, we present a comparative analysis of the metabolic state of embryonic organs at mid to late gestational stages using a diabetic pregnancy mouse model. Although glucose uptake drastically decreases during mid-gestational stages in the developing heart during normal pregnancy, during diabetic pregnancy, the embryonic hearts maintain high glucose uptake and high glycolytic activity until the late gestational stages. Higher glycolytic activity is accompanied by alterations in the levels of metabolites in TCA cycle and nucleotide biosynthesis: α-ketoglutarate to succinate ratio remained high, leading to the decrease in H3K36me3 levels possibly due to higher activity of Kdm2. The tri- and di-phosphate nucleotides to monophosphate nucleotides ratio was also significantly increased, resulting in dysregulation of mTOR signaling and decreased activity of AMPKα. The supplementation of cell-permeable α-ketoglutarate and AICAR promotes and suppresses cardiac maturation in vitro, respectively, suggesting that disproportional changes in downstream metabolites impair cardiac maturation in diabetic pregnancy. These data suggest a previously underappreciated causative link between the aberrant metabolites and cardiac gene regulatory network as a pathomechanism of congenital heart disease in diabetic pregnancies.

Funding Source: NIH R01 HL142801 Rose Hills Foundation Award, UCLA BSCRC
Keywords: metabolism, cardiac development, diabetic pregnancy

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GENERATION OF VALVULAR INTERSTITIAL CELLS FROM HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOCARDIAL CELLS

Mikryukov, Alexander - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*
Mazine, Amine - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*
Latifi, Neda - *Translational Biology and Engineering Program, The Ted Rogers Center for Heart Research, Toronto, ON, Canada*
Wei, Bei - *Department of Genetics, Stanford University, Stanford, CA, USA*
Yang, Donghe - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*
Miao, Yifei - *Center for Stem Cell and Organoid Medicine, CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Gu, Mingxia - *Center for Stem Cell and Organoid Medicine, CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Simmons, Craig - *Translational Biology and Engineering Program, The Ted Rogers Center for Heart Research, Toronto, ON, Canada*
Keller, Gordon - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*

Abstract: Heart valve defects are present in ~1/3 of congenital heart disease cases, the most common form of human birth defects. Many affected individuals will require valve replacement. Generation of various valve cell populations in vitro from human pluripotent stem cells (hPSC) would provide unique opportunities to study human valve disease as well as engineer biological valves. During embryonic development the cell populations comprising heart valves derive from endocardium, a specialized endothelial cell population that lines the chambers of the heart and induces the formation of the first functional population of cardiomyocytes, known as trabecular cardiomyocytes. To access human endocardial cells, we developed a protocol that promotes the generation of NKX2-5+CD31+ endothelial cells from hPSC-derived cardiovascular progenitors by manipulating BMP10 signaling. These cells express the cohort of genes that identifies the endocardial lineage in vivo and show a high level of transcriptional similarity to primary human fetal endocardium. Furthermore, these hPSC-derived NKX2-5+CD31+ endocardial-like cells display the ability to induce a trabecular fate following co-culture with target cardiomyocytes, and the capacity to undergo EndoMT to give rise to mesenchymal cells that express markers of valvular interstitial cells (VICs). Further culturing of these VIC-like cells resulted in their segregation into two subpopulations secreting different amounts of collagens and proteoglycans/glycosaminoglycans, recapitulating the heterogeneity observed in the stratified extracellular matrix of normal valves. In summary, the findings presented in this report describe a method for the derivation of valvular interstitial cells from hPSCs.

Funding Source: This work was supported by grants from the Canadian Institute of Health Research (PJT148717, PJT156432, and FDN159937).
Keywords: Heart valves, Cardiac differentiation, Endocardium

TOPIC: EARLY EMBRYO

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DERIVATION OF AXIAL LINEAGES FROM HUMAN PSCS

Kee, Nigel - *Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*
Petipre, Charles - *Neuroscience, Karolinska Institutet, Stockholm, Sweden*
Hagey, Daniel - *Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden*
Benlefki, Salim - *Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*
Mei, Irene - *Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*
Alcade, Silvia - *Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*
Dias, Jose - *Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*

Nijssen, Jik - *Molecular Cell Biology and Genetics, Max Planck Institute, Dresden, Germany*
Lallemend, Francois - *Neuroscience, Karolinska Institutet, Stockholm, Sweden*
EL Andaloussi, Samir - *Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden*
Ericsson, Johan - *Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*
Hedlund, Eva - *Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

Abstract: Axial stem cells (ASC) reside in a small niche located at the caudal extent of the post-gastrulation embryo. Here they give rise to axial tissues including post-cranial spinal cord, neural crest and somitic mesodermal lineages. In vitro models recreate the ASC niche by mimicking key morphogenic patterning pathways including WNT and FGF signalling cascades. Specification of downstream lineages requires subsequent modulation of SMAD, SHH and RA pathways, among others. We have developed new protocols to derive ASCs and their daughter lineages from human PSCs. We find that careful and balanced control of multiple signalling pathways is required to specify stable ASCs that maintain multi-potency. Our ASCs can be directed down any of the three daughter lineages using mutually exclusive morphogen paradigms. Neural lineages respond to dorso-ventral patterning cues, and also exhibit co-linear HOX activation in line with the timing of their exit from the ASC state. These protocols will serve as a valuable platform for the modelling of development of human axial tissues, and the diseases that affect them.

Keywords: Neuromesodermal, HOX, Axial

TOPIC: EPITHELIAL_GUT

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GASTRIC CANCER PROGRESSION INDUCED BY HELICOBACTOR PYLORI INFECTION IN HUMAN GASTRIC CANCER ORGANOIDS

Yokoyama, Kazushige K. - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Wuputra, Kenly - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Pan, Jia-Bin - *Gradutae Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Ku, Chia-Chen - *Gradute Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Liu, Chung-Jung - *Gastroenterology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
Lin, Chang-Sheng - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Wu, Deng-Chyang - *Gatsroenterology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*

Abstract: The gastric cancer- and normal-organoids from the cancerous parts and iPS cells were established several years ago. Owing to the remarkable degree to which they recreate the cellular diversity observed in the human stomachs, they have attracted significant interest as a novel model system for precision medicine. However, many questions remain about the extent to which these cultures recapitulate gastro development and mechanism of Helicobacter pylori infected cancer progression. The effect of the growth factors for cancer progression by Helicobacter pylori on stomach organoids were examined. Previously, we reported that the Hepatocyte derived growth factor (HGDF) and TNF-alpha dependent pathways were crucial for cancer development using 2-D cells. Here, we identified the heterogeneity of the progression of cancers derived from human gastric organoids in response to the Hepatocyte derived growth factor (HGDF) and TNF-alpha (TNFα) dependent pathways during the infection by Helicobacter pylori on stomach organoids. TNFα is defined as the upstream partner of HGDF in 2-D organoids culture system, however, in 3-D organoids culture, HDGF and TNFα are independent pathways in the infection of Helicobacter pylori. The invasion activity induced by Helicobacter pylori was inhibited by TNFα, but not by HDGF. We will discuss that this difference is due to the heterogeneity of the signaling between 2-D and 3-D cultivation system.

Funding Source: Ministry of Science and Technology (110-2314-B-037-141; 110-2320-B-037-028), National Health Research Institutes (EX109-10720SI), Kaohsiung Medical University (KMU-M106001; KMU-TC108A02), and (SA10803C; KMUH 110-0R86).
Keywords: Helicobacter pylori, Organoid, Stomach cancer

TOPIC: EYE AND RETINA

730

RD3 INTERACTS WITH NUCLEAR SUB-COMPARTMENT PROTEINS AND REGULATE C/D BOX SMALL NUCLEOLAR RNA STABILITY AND FUNCTION

Mahato, Sudipta - *Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India*
Maddileti, Savitri - *Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, Hyderabad, India*
Pulimamidi, Vinay Kumar - *Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, Hyderabad, India*
Susaimanickam, Praveen Joseph - *Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, Hyderabad, India*
Mariappan, Indumathi - *Centre for Ocular Regeneration, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, Hyderabad, India*

Abstract: Retinal Degeneration 3 gene encodes a 23 kDa scaffolding protein that mediates the trafficking of retinal guanylyl cyclase (RetGCs) from the inner to outer segments of photoreceptors. Mutations in RD3 cause Leber Congenital Amaurosis, Type 12 (LCA12), a severe form of congenital retinal dystrophy resulting in early-onset vision loss in young children below 5 years of age. Ectopically expressed RD3 localizes to discrete punctate structures both in the cytoplasm and nucleus. While it interacts with GCs and mediates their intracellular trafficking in the cytoplasm, the role(s) of nuclear RD3 remains unknown. To study the role of nuclear RD3 and its effects on retinal lineage differentiation, iPSC lines were derived from patient-specific dermal fibroblasts. Upon differentiation, both the healthy and patient-specific iPSCs formed normal appearing eye fields and generated retinal cups at comparable timelines and efficiencies, which suggest that the early retinal commitment remains unaffected in mutant cells. Global gene expression analysis of iPSC-derived retinal cups revealed significant downregulation of several small non-coding RNAs belonging to the C/D box SNORD 113 and 114 families in patient-specific tissues. Further, immunopulldown, mass spec-

trometry, and western blot analysis revealed interactions of RD3 with multiple nuclear sub-compartment proteins and RNA binding proteins. Co-localization studies have confirmed that the nuclear RD3 puncta perfectly overlap with the PML bodies and a subset of them co-localize with the Cajal bodies and NOP58 containing nucleolar compartments. RNA immunoprecipitation and RT-PCR analysis confirmed the presence of a select few SNORDs in the wild-type RD3 bound ribonucleoprotein complexes. These observations together suggest the possible involvement of RD3 in small nuclear RNA stabilization, maturation, or intranuclear trafficking and may indirectly regulate various cellular processes, such as RNA modifications and splicing; ribosomal biogenesis, and protein synthesis.

Funding Source: Department of Biotechnology, Ministry of Science & Technology, Government of India
Keywords: RD3, LCA12, retinal degeneration, iPSC, retinal cups, SNORDs, C/D Box SNORDs, NOP58, Cajal bodies

TOPIC: IMMUNE SYSTEM

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ACTIVATION-DEPENDENT INDUCTION OF THE NLRP3 INFLAMMASOME IN HUMAN IPSC-DERIVED MICROGLIA

Mathews-Ajendra, Mona A. - *Cellomics, Life & Brain GmbH, Bonn, North Rhine-Westphalia, Germany*
Besemer, Brenda - *Institute of Reconstructive Neurobiology, University of Bonn, Germany*
Frickel, Vanessa - *Cellomics, Life & Brain, Bonn, Germany*
Brüstle, Oliver - *Institute of Reconstructive Neurobiology, University of Bonn, Germany*

Abstract: Neuroinflammation is a key feature of several neurodegenerative disorders. Microglia, the brain-resident immune cells, are a major contributor to this process. For example pathological microglia can exhibit constitutive activation of the NLRP3 inflammasome pathway. We developed an in vitro stem-cell based human microglia model for probing the activation of NLRP3 inflammasome pathway. Using this model, assays to validate the NLRP3 inflammasome pathway as well as its upstream target NFkb and downstream targets caspase 1 and IL1-beta (IL1b) were developed. Canonical induction of the NLRP3 inflammasome in microglia requires a two-step process of priming followed by activation to induce the oligomerization of NLRP3 and recruitment of ASC to form specks. Consequently pyroptotic proteins caspase 1 and inflammatory proteins IL1b and IL18, which together lead to cell death and inflammation, are activated. Priming of our iPSdMiG with LPS followed by activation with nigericin reproducibly induced the formation of ASC specks and caspase 1-driven cell death. ASC speck formation induced by LPS/nigericin stimulation was completely ablated by NLRP3-specific inhibitor MCC950. However, targeting the NLRP3 activator NFkB using MG-132, a broad proteasome inhibitor, led only to a partially reduced inflammasome activation. We then explored whether aggregated amyloid beta (A-beta) can prime or activate the NLRP3 inflammasome. A-beta alone was not sufficient to induce microglial priming or ASC speck formation. However, primed microglia that was then exposed to A-beta induced ASC speck formation. As seen with nigericin activation, A-beta induced ASC specks were completely ablated by MCC950, and only partially reduced by MG-132. However, time dynamics, quantity of ASC-speck formation and downstream effects differed between the two activation stimuli. Primed iPSdMiG activated with A-beta responded faster (30 minutes post activation) but with fewer ASC-speck forming cells (avg. 5 %) compared to activation with nigericin (2 hours / avg. 15 %). Our in vitro cellular model of human microglia with validated inflammasome pathway-based read-outs will provide mechanistic insight required for deciphering inflammasome dynamics in response to disease-relevant stimuli such as A-beta and thus support therapy development.

Keywords: iPS cell-derived microglia, NLRP3 inflammasome, amyloid beta

TOPIC: LIVER

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LOSS-OF-FUNCTION MUTATION OF KIF3B CAN CAUSE A DEFECTIVE BILIARY DEVELOPMENT IN BILIARY ATRESIA: EVIDENCE FROM IPSC-DERIVED BILIARY ORGANOID

Liu, Hailong - *Surgery, The University of Hong Kong, Hong Kong*
Tang, Clara Sze Man - *Surgery, the University of Hong Kong, Hong Kong*
Tam, Paul Kwong Hang - *Surgery, the University of Hong Kong, Hong Kong*
Lui, Vincent Chi Hang - *Surgery, the University of Hong Kong, Hong Kong*

Abstract: Biliary Atresia is a poorly understood devastating fibro-obliterative biliary disease of newborns. Limited access to primary biliary tissue, difficulties in culturing primary biliary cells (cholangiocytes) and inadequate animal disease model have led to a slow advancement in unravelling the patho-mechanisms, in diagnosis and treatment for BA. Human iPSC-derived biliary organoids provide us an unprecedented cellular model to study the patho-mechanisms for BA. We have conducted whole exome sequencing on 85 BA trios, identified deleterious de novo variants in liver-expressed ciliary genes including KIF3B in 31.5% BA patients, and demonstrated absence of cilia in the BA livers with KIF3B LOF (loss-of-function) mutations. KIF3B (Kinesin Family Member 3B) encodes protein that is a subunit of the anterograde IFT (Intraflagellar transport) motor protein kinesin II, and disruption of the IFT impairs cilia formation/functioning. Cholangiocyte has a single cilia, which functions physiologically as cellular antennae to detect and transmit signals that influence cholangiocyte function/development. In this study, we generated KIF3B+/- & KIF3B -/- human iPSC cells, and differentiated them into biliary organoids to investigate the impacts of the KIF3B LOF in biliary development in BA. Immuno-staining for markers for definitive endoderm, hepatoblast, cholangiocyte progenitors and cholangiocytes was performed on differentiating normal and KIF3B mutant iPSCs. We showed that KIF3B+/- and KIF3B -/- iPSCs are less capable in the generation of hepatoblast and cholangiocyte progenitors. Furthermore, KIF3B+/- & KIF3B -/- biliary organoids were few, tiny and with abnormal or no cilia. Expression of SOX9 and CK19 (cholangiocyte markers) was decreased in KIF3B +/- and KIF3B-/- organoids. Single-cell RNA sequencing analysis are in progress to identify signaling pathways underlying the defective biliary development in KIF3B mutants. Taken together, our data show that KIF3B play a key role in cholangiocyte and biliary development, and demonstrate that the human iPSC-derived biliary organoid is a valuable disease model for patho-mechanistic study of BA.

Keywords: iPSC-derived biliary organoid, KIF3B, Biliary Atresia

TOPIC: NEURAL

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PLURIPOTENT STEM CELLS DERIVED INNER EAR ORGANOIDS RECAPITULATE OTIC DEVELOPMENT IN VITRO

Roccio, Marta - *Department of Otorhinolaryngology, Head and Neck Surgery, University of Zurich, Switzerland*
Doda, Daniela - *ORL, Head and Neck Surgery, University of Zurich, Switzerland*
Valsamides, Victoria - *ORL, Head and Neck Surgery, University of Zurich, Switzerland*

Abstract: Inner ear hair cells and auditory neurons are essential for sound detection. Their damage or loss is irreversible in humans and is a major cause of hearing deficit. Stem cell-based models open new opportunities to understand the pathophysiology of hearing loss and to develop novel therapeutics. Furthermore, they could provide new means to gain insight into inner ear development. Inner ear morphogenesis can be in part recapitulated using pluripotent stem cell directed differentiation in inner ear organoids (IEOs). This step-wise differentiation protocol consists of 3 main steps: otic placode differentiation, otic vesicle formation and maturation. Multiplex immunostaining was used to characterize the differentiation of human iPSC lines in IEOs. We further compared early stages of IEO development with human embryos at Carnegie Stages (CS) 11, 12 and 13. Simultaneous inhibition of TGFb and activation of BMP signaling was shown to be sufficient to specify non neural ectoderm/cranial placode at the surface of IPSC aggregates. Subsequent BMP inhibition and activation of FGF signaling resulted in otic placode differentiation by day 8-12 of culture. Titration of BMP4 revealed optimal levels required for specification of placodal tissue co-expressing ECAD/AP2/SIX1/NCAD and by day 8, the otic marker PAX8. Similar marker expression was observed in CS11 human embryos. Following induction of placodal fate, IEOs were incubated in presence of the Wnt agonist CHIR99021. This led to further development of otic vesicle-like structure expressing SOX2/PAX2/PAX8/ECAD/FBXO2 and SOX10. In vitro derived otic vesicles at day 30-40 of culture showed remarkable similarity to CS13 embryos. Finally, starting from day 55, the differentiation of inner ear specific sensory epithelia was observed. Sensory hair cells (MYO7A/POU4F3/ESPIN positive) developed intercalated to SOX2 supporting cells and received innervation from co-differentiated otic-like neurons, expressing TUBB3/SOX2/BRN3A and ISL1/2. Ongoing studies are focusing on the molecular and functional characterization of these cell types by transcriptional profiling and drug-sensitivity assays. The establishment of robust differentiation methods, benchmarked against human tissue, provides a unique tool to expand our knowledge on human development.

Keywords: Inner ear organoids, directed differentiation, sensory organs

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MODULATION OF THE P75 PAN-NEUROTROPHIN RECEPTOR ALTERS ADULT NEUROGENESIS AND CONSTITUTES A THERAPEUTIC TARGET IN ALZHEIMER'S DISEASE

Chanoumidou, Konstantina - *School of Medicine, University of Crete, Heraklion, Greece*
Papadopoulou, Marianna - *University of Crete, School of Medicine, Heraklion, Greece*
Charalampopoulos, Ioannis - *University of Crete, School of Medicine, Heraklion, Greece*

Abstract: The pan-neurotrophin p75 receptor (p75NTR) is a member of the TNF death receptor superfamily with pleiotropic expression in neural tissue and multifunctional regulatory role ranging from neural plasticity to cell death. Its up-regulated expression in neurodegenerative conditions combined with its controversial signaling in different cellular settings as pro-apoptotic and/or pro-survival mediator, makes it an appealing target in neuropathology. p75NTR has been linked with Alzheimer's Disease (AD) by serving as a receptor for amyloid-β (aβ), the major component of the amyloid plaques found in the brain of AD patients, while it is involved in neuronal cell death. However, its contribution to adult hippocampal neurogenesis, which drops sharply in AD, has not been clarified yet. Here, we aim to investigate the role of p75NTR in neurogenesis by addressing its function in primary adult hippocampal and human iPSC-derived neural stem cells (NSC) under physiological and AD related conditions. p75 KO mice present with decreased NSC proliferation and attenuated neuronal differentiation in the dentate gyrus as shown with immunohistohemistry analyses for DCX and NeuN revealing key neurogenic properties of p75NTR. Additionally, p75NTR signaling is active in human iPSC-derived NSC and becomes deregulated in cells derived from AD patients bearing the ApoE4 mutation. Finally, p75NTR regulates NSC survival in the presence of aβ peptides indicating its involvement in the neurogenic deficits reported in AD. Configuration of the p75NTR signaling that regulates neurogenesis and its pharmacological targeting will enable the enhancement of the endogenous repairing ability against AD-related neuronal loss introducing new neuroprotective and neurorestorative therapies.

Funding Source: Hellenic Foundation for Research and Innovation "1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment" (Pr. Nu.:HFRI-FM17-2301) (ELKE UoC:KA10490)
Keywords: p75NTR, Alzheimer's Disease, Neural Stem Cells

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LONGITUDINAL CHARACTERIZATION OF SPONTANEOUS CALCIUM TRANSIENTS IN FOREBRAIN ORGANOIDS USING A GENETICALLY-ENCODED CALCIUM INDICATOR

Harary, Paul - *Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Rahaman, Imon - *Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Jgamadze, Dennis - *Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Song, Hongjun - *Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Ming, Guo-li - *Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Chen, H. Isaac - *Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Abstract: Assessing the functional activity of cerebral organoids is critical to creating models that better recapitulate native brain tissue. Several recent investigations have used calcium-imaging and extracellular recordings to assess cerebral organoids, but there have been few detailed longitudinal studies of the activity of maturing organoids. Here, a genetically encoded calcium indicator was used to acquire repeated recordings of the same organoid samples throughout maturation. Forebrain organoids were grown from an induced pluripotent stem cell line (C1.2) derived from a healthy volunteer using a previously published differentiation protocol. At differentiation day (dd) 40, organoids were sliced at a thickness of 500µm. Viral transduction was performed on dd58 with AAV expressing GCaMP8m under a CAG promoter at ~7×1010 vg/mL. Calcium activity was measured at dd70, 130 and 150 and analysis of images was performed using the Minian pipeline and additional custom scripts. During maturation, organoids demonstrated an increased number of bursting events, decreased variability in firing rates among regions, and increased number of synchronous clusters. Global synchrony was observed across whole organoids in BrainPhys-based medium (BP) as indicated by synchronized calcium transients and inferred spiking events. In contrast to BP, multiple independent synchronous clusters were observed in Neurobasal-based medium (NB). This study reveals longitudinal differences in spontaneous activity and synchronicity of the same organoid samples at different maturational stages. BP produced increased global synchrony as compared to NB. Optical imaging provides reduced sampling bias and is non-terminal as compared to acute in vitro electrophysiology. Importantly, these recordings were taken from intact organoids cultured in suspension. This avoids perturbation of morphology that may result from long-term culture on a planar multi-electrode array, an alternative approach to acquiring longitudinal data. This method may be used to select highly functional organoids for transplantation studies and better inform in vivo development post-transplant. Furthermore, functional analysis of intrinsic organoid activity provides a valuable model of human neurodevelopment and disease-specific phenotypes.

Funding Source: National Institutes of Health (R01-NS119472 to H.I.C)
Keywords: Cerebral organoids, Calcium imaging, Neurodevelopment

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GATAD2B-ASSOCIATED NEURODEVELOPMENTAL DISORDER AND THE NURD COMPLEX: IPSC MODELING OF NEURODEVELOPMENT

Pierson, Tyler Mark - *Pedatrics, Neurology, and Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Otero, Maria Gabriela - *Regenerative Medicine Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA*

Abstract: GATAD2B is a subunit of the NuRD complex, which is involved with differentiation, reprogramming, and neuronogenesis. GATAD2B-associated Neurodevelopmental Disorder (GAND) presents with macrocephaly, intellectual disability, apraxia of speech and cortical dysfunction that is linked to loss-of function and missense variants. We generated five patient-derived IPSC lines from GAND- and CTL-subjects each. GAND- and CTL-IPSCs were differentiated into Nestin+ NPCs and LoF-GAND IPSCs and NPCs showed GATAD2B mRNA and protein levels were ~50% of normal (consistent with haploinsufficiency). Bulk RNA-seq analysis of GAND- vs. CTL-lines identified ~650 differentially-expressed genes (DEGs) in NPC cultures, but only 6 DEGs in IPSC cultures indicating that GATAD2B played a greater role in NPCs than IPSCs. Upregulated gene-ontology-pathway sets in GAND-NPCs included “Forebrain-Cell-Migration,” “Cerebral-Differentiation-Neuron,” and “Cerebral-Cortex-Development;” down-regulated sets included “DNA-Replication-Dependent-Nucleosome-Organization” and “Chromatin-Silencing.” Some of the most up-regulated DEGs included genes that play an important role in corticogenesis, with ARX being most significant. Interestingly, Gatad2b was identified as an upregulated DEG in Arx-KO mouse cortices and previous work showed Arx-overexpression in progenitors prolonged cell-cycle progression and slowed cell growth. Subsequent studies showed GAND-NPCs had growth curves over 24-48 hours were significantly decreased by ~20%. NPCs were further differentiated into cortical neurons (CNs) and evaluated for sequential expression of cortical markers. CN-cultures generated similar numbers of TUJ1/MAP2ab neurons; however, GAND-CNs had altered CTIP2-SATB2 patterns in relation to each other and over time. SATB2 appeared early and was expressed concurrently with CTIP2 in GAND-CNs. The GAND mouse also had abnormal cortical patterning, with increased numbers of co-expressing Ctip2+Satb2+ GAND-CNs. These results indicate that GAND-IPSCs are valid models of GATAD2B/Gatad2b deficiency’s impact on gene expression and NPC/CN populations.

Keywords: GATAD2B, NuRD Complex, Corticogenesis

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EXPLORING A HIPSC-DERIVED MODEL TO ADDRESS GLIAL ACTIVATION IN NEUROINFLAMMATION

Gomes, Catarina Monteiro - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
Gonçalves, Carolina - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
Perdigão, Catarina - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
Silva, Gabriela - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
Simão, Daniel - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
Brito, Catarina - *Animal Cell Technology Unit, iBET - Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

Abstract: Neuroinflammation is associated with pathogenic processes and disease states. Upon inflammatory stimuli, glial cell activation contributes to tissue healing and to restore the central nervous system (CNS) homeostasis. However, excessive glial activation causes neuronal death and chronic neuroinflammation. Still, the molecular mechanisms that trigger and sustain glial activation are poorly described. Experimental models in which the human neural cells and their microenvironment are represented will be key to study such processes. This work aimed to establish a human cell model of neuroinflammation to dissect cellular crosstalk along the neuroinflammatory axis. We employed a methodology pioneered by our team, in which human-induced pluripotent stem cell (hiPSC)-derived neural progenitors are cul-

tured in perfusion stirred-tank bioreactors and differentiated into 3D neurospheroids composed of neurons, astrocytes, and oligodendrocytes. To recapitulate the neuro-immune axis, a co-culture of neurospheroids and hiPSC-derived microglia (iMGL) was implemented. iMGL infiltrated the neurospheroid 3D structure, adopted a ramified phenotype, and maintained the expression of typical microglia markers (TMEM119, TREM2, and IBA1). Neurospheroids with or without iMGL were challenged with neuroinflammatory factors reported to induce activation of astrocytes in mice models, namely TNF-α, IL-1α, and C1q. Upon inflammatory challenge, neurospheroids showed upregulation of neuroinflammatory genes (e.g., SERPINA3, and C3), concomitant with secretion of proinflammatory cytokines and chemokines (e.g., CXCL8/IL8, and CCL2). Quantitative transcriptomic (NGS) and proteomic (SWATH-MS) functional enrichment analysis highlighted the activation of NF-κB signaling upon challenge. Altogether these results indicate that astrocytes within the neurospheroids undergo canonical astrogliosis events associated with TNF-α exposure, hallmarks of neuroinflammation. Ongoing work focuses on characterizing the neuron-glial interactions under a neuroinflammatory microenvironment. Hence, we propose the human neurospheroid model as a useful tool to explore neuroinflammatory mechanisms.

Funding Source: FCT/MCTES: iNOVA4Health (UIDB/04462/2020; UIDP/04462/2020), PTDC/BTM-ORG/29580/2017 and UI/BD/151253/2021 (CMG PhD fellowship). EU/EFPIA/Innovative Medicines Joint Undertaking ARDAT grant No 945473.
Keywords: Neuroinflammation, Innate Immunity, Glial Activation

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A CELL BASED ASSAY FOR THE DETECTION OF TETANUS TOXIN

Lindsay-Hill, Luke - *UK Stem Cell Bank, National Institute for Biological Standards and Control, Potters Bar, UK*
Stickings, Paul - *Bacteriology, National Institute of Biological Standards and Control, Hertfordshire, UK*
Mattioli, Elisa - *UK Stem Cell Bank, National Institute of Biological Standards and Control, Hertfordshire, UK*
Rajagopal, Shalini - *Bacteriology, National Institute of Biological Standards and Control, Hertfordshire, UK*
Ware-Cornish, Kate - *UK Stem Cell Bank, National Institute of Biological Standards and Control, Hertfordshire, UK*

Abstract: Tetanus neurotoxin (TeNT) is produced by the bacterium Clostridium tetani. It exerts its toxic effect through cleavage of the synaptic protein vesicle-associated membrane protein (VAMP2) in spinal cord interneurons. This prevents neurotransmitter release leading to a loss of inhibitory input to motor neurons. The resultant overexcitation leads to severe spasms typical of the disease. Tetanus vaccines are comprised of chemically inactivated toxin (toxoid) and therefore each batch must be tested for the absence of toxin and irreversibility of toxoid. Current safety assays for tetanus vaccines employ guinea pigs or mice, with no alternative cell-based assays that are suitably specific or sensitive. We report a human cell-based assay using human embryonic stem cell (hESC) derived neurons capable of detecting nanomolar concentrations of TeNT. We demonstrated VAMP2 protein expression in our neurons seven days after terminal neuronal differentiation, which was cleavable in a dose dependent manner with TeNT. Following optimisation of assay conditions total VAMP2 cleavage was achieved following a 24hr 1nM TeNT treatment. We further characterised our model demonstrating selective uptake of TeNT into our neurons using a TeNT - Botulinum B fusion toxin. Scaling up and translating our assay into the field will require cells shipping as cryopreserved progenitors and operators performing the final post-mitotic differentiation. Therefore, we analysed progenitor viability and differentiation efficiency post cryopreservation. We did note a reduction (~20%) in viability post-thaw over long term storage in liquid nitrogen (>3 months). A screen of agents with known neuroprotective properties added to the cryopreservation media identified FGF2 (20ng/ml), which mitigated the loss of viability following cryopreservation. Importantly, long term cryopreservation did not affect terminal differentiation of progenitors into neurons or their sensitivity to TeNT. We report a human cell-based assay for the detection of tetanus toxin. Further optimisation and characterisation is now underway to increase the sensitivity of the assay and determine the feasibility of widespread adoption in the field and final regulatory acceptance.

Keywords: Tetanus vaccine, Cell-based assay, Safety assay

TOPIC: PANCREAS

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ARGININE 65 METHYLATION OF NEUROGENIN 3 BY PRMT1 IS A CRITICAL FOR DEVELOPMENT OF HESCS INTO PANCREATIC ENDOCRINE CELLS

Gahyang, Cho - *Biosciences, KAIST, Yuseong-gu, Korea*
Han, Yong-mahn - *Biosciences, KAIST, Yuseong, Korea*

Abstract: Neurogenin3 (NGN3) is a crucial transcription factor in the cell fate determination of endocrine progenitors (EPs) in the developing pancreas. Although the activation and stability of NGN3 are regulated by phosphorylation, the role of arginine methylation of NGN3 is poorly understood. Here, we report that arginine 65 methylation of NGN3 is absolutely required for the pancreatic lineage development of human embryonic stem cells (hESCs) in vitro. During pancreatic EC differentiation, inducible knock out of the protein arginine methyltransferase-1 (PRMT1) impaired EC development from EPs. PRMT1 is a predominant arginine methyltransferase in mammalian cells. Loss of PRMT1 caused an accumulation of NGN3 in the cytoplasm of EPs and blocked NGN3's transcriptional activity in PRMT1-KO EPs. We also found that PRMT1 specifically methylates NGN3 arginine 65, and this modification is a prerequisite for ubiquitin-mediated NGN3 degradation. Our findings indicate that arginine 65 methylation of NGN3 is a critical molecular switch in hESCs in vitro, permitting the differentiation of pancreatic endocrine lineages.

Funding Source: This study was supported by a National Research Foundation (NRF) 2015M3A9B302821823 and 21A0402L1-11, Republic of Korea.
Keywords: PRMT1, Neurogenin3, pancreas development, Inducible knockout system, CRISPR

TOPIC: PLURIPOTENT STEM CELLS

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INVESTIGATING PATHOPHYSIOLOGY OF USHER SYNDROME TYPE 1B USING INNER EAR ORGANOIDS

Connolly, Keeva - *Stem Cell Medicine Group, Children's Medical Research Institute, Westmead, Australia*
Gonzalez-Cordero, Anai - *Stem Cell Medicine Group, Children's Medical Research Institute, Westmead, Australia*
Lee, Scott - *Stem Cell and Organoid Facility, Children's Medical Research Institute, Westmead, Australia*

Abstract: Usher syndrome is the leading inherited cause of deaf-blindness. Mutations in MYO7A, which encodes the unconventional motor protein Myosin VIIA, have been associated with Usher syndrome type 1b. Hallmarks of Usher syndrome type 1 include severe-to-profound deafness at birth, chronic balance problems and vision loss progressing from childhood. The former two symptoms are caused by dysfunctional sensory hair cells in the cochlea and vestibular apparatus of the inner ear. It has been predicted that Myosin VIIA interacts with other Usher proteins during hair cell development and in mechanotransduction, where physical stimuli (soundwaves or movement) are converted into electrical impulses. In order to study Myosin VIIA in the human otic system, inner ear organoids have been generated from induced pluripotent stem cells (iPSCs) derived from an Usher1b patient and from an isogenic control iPSC line. Stem cells were successfully differentiated using a published stepwise protocol where addition of exogenous factors mimics in vivo activation and inhibition of signalling pathways involved in normal otic development. The resulting organoids contain sensory epithelia-like structures comprising hair cells, supporting cells and innervating neurons. Immunohistochemistry at multiple time points during organoid development has enabled comparison of otic development and hair cell-specific marker expression in diseased and control cell lines. A comprehensive comparison of expression and spatial distribution of Usher proteins will characterise and confirm Myosin VIIA involvement in Usher protein interactions during development. This study will help to elucidate disease mechanisms as well as the role of Usher proteins in human hair bundle development and functionality.

Keywords: Inner ear organoids, Disease modelling, Usher syndrome

780

ACTIVATION OF HERV-K(HML-2) DISRUPTS CORTICAL PATTERNING AND NEURONAL DIFFERENTIATION BY INCREASING NTRK3

Nair, Vidya - *Department of Virology, Helmholtz Center Munich, Neuherberg, Germany*
Liu, Hengyuan - *Department of Genome-Oriented Bioinformatics, TUM, Munich, Germany*
Ciceri, Gabriele - *Developmental Biology and Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Jungverdorben, Johannes - *Developmental Biology and Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Frishman, Goar - *Institute of Experimental Genetics, Helmholtz Zentrum Munich, Munich, Germany*
Meyer, Jens - *Institute of Human Genetics, University of Saarland, Homburg, Germany*
Hadian, Kamyar - *Assay Development and Screening Platform, Helmholtz Zentrum Munich, Germany*
Studer, Lorenz - *Developmental Biology and Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Vincendeau, Michelle - *Institute of Virology, Helmholtz Zentrum Munich, Germany*

Abstract: Understanding the impact of the biological function and disease association of human endogenous retroviruses (HERVs) remains one of the most challenging and largely elusive area of scientific research. HERV-K(HML-2) has been reported to be associated with neurotoxicity, but no clear understanding of its functional role or mechanistic basis has been conclusively concluded so far. Here, in our study, we have attempted to address the physiological significance of HERV-K(HML-2) in neuronal differentiation using CRISPR engineering in a human-pluripotent-stem-cell-based system. We found that elevated HERV-K(HML-2) transcription is detrimental for the development and functionality of cortical neurons. HERV-K(HML-2) overexpressing cortical neurons showed a clear reduction in the neuronal marker MAP2 after differentiation into cortical neurons and almost completely lost their functional status. In contrast, transcriptional activation of HERV-K(HML-2) LTRs during differentiation into dopaminergic neurons had no effects on MAP2 levels, suggesting a cell-type-specific pattern of action. Moreover, high HERV-K(HML-2) transcription altered the cortical layer formation in forebrain organoids, resulting in a disruption of cortical patterning. HERV-K(HML-2) transcriptional activation leads to hyper activation of specific cellular genes, including the developmental factor NTRK3, implicated in neurodegeneration supporting the discovery that HERV-K(HML-2) transcriptional activation negatively impacts cortical development. Direct activation of NTRK3 phenotypically resembles HERV-K(HML-2) induction, and a reduction of NTRK3 levels in context of HERV-K(HML-2) induction restores cortical neuron differentiation. Hence, these findings demonstrate that activation of the endogenous retrovirus group HERV-K(HML-2) negatively impacts cortical neuronal development by activating the classical developmental factor NTRK3. In summary, this study shows that the tight regulation of HERV-K(HML-2) is detrimental for healthy brain development and that dysregulation of these elements may be associated with neurodegenerative diseases.

Keywords: Retrotransposons, Human Endogenous Retroviruses and CRISPR, Cortical Neurons Forebrain organoids

TOPIC: NEURAL

784

ESTABLISHING NEUROSPHERES WITH MESENCHYMAL STEM CELLS IN A 3D SUSPENSION CULTURE SYSTEM TO OPTIMIZE NEURITE GROWTH AND FACILITATE NERVE REPAIR

Harley-Troxell, Meaghan E. - *LACS, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*
Millet, Larry - *Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN, USA*
Newby, Steven - *Large Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*
Anderson, David - *Large Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*
Dhar, Madhu - *Large Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA*

Abstract: The overall goal of this research is to promote nerve repair through applications of carbon-based nanoparticles and mesenchymal stem cells (MSCs) in a synthetic extracellular matrix. We hypothesize that the combination of carbon nanocomposites and MSCs in nerve tissue engineering strategies will provide us with the optimal environment for neuronal growth. Towards this goal, we first validated neural markers including, Vimentin, Microtubule Associated Protein 2, β-Tubulin III, and Neuron-Specific Enolase which were expressed by the MSCs as they underwent neural differentiation in vitro. Next, we established neurospheres in vitro using primary neurons isolated from 1 to 3-day old rat hippocampi. We achieved neurospheres containing >100 µm long neurites, with a cluster density of >50 µm, providing an optimal cell-to-cell communication. Subsequently, we incorporated ex vivo expanded and previously characterized rat bone marrow-derived MSCs with neurospheres. These constructs were established via a new commercial, high throughput 3D culture system in which a single 3D bioreactor provides a stress-free environment. Experiments to compare freshly expanded versus cryopreserved MSCs using two ratios of MSCs: primary neural cells: 10:90, and 50:50 on two special substrates (laminin and poly-D-lysine (PDL)) were carried out. Tissue culture treated polystyrene surface was used as a control. The cultures were evaluated for neurosphere size and neurite length using phase contrast microscopy, followed by Image J analysis. Cell viability was evaluated using 0.4% trypan blue. The data shows that laminin provides superior neurite outgrowth for neurospheres compared to non-biological (TCPS) and conventional (PDL) synthetic substrates. Both ratios showed >100 µm neurite length and >50 µm cluster density with varied viability, suggesting that the ratios of the two types of cells need further analysis. Our next steps are to incorporate endothelial cell populations to develop an in vitro organoid for neuroregenerative studies. Identity of each cell type will be assessed using cell-specific protein markers. The long-term goal is to evaluate neuro-organoids in rat models of traumatic brain injuries (TBI).

Funding Source: This research is supported by NIH-NIBIB grant number R03EB028494 (LJM), grants from the University of Tennessee Office of Research (DEA), and NIH-NIAMS grant number R15 AR070460-01 (MD).
Keywords: Nerve Repair, 3D Co-Culture, Mesenchymal Stem Cells

TOPIC: LIVER

786

DEVELOPMENT OF THE MITOCHONDRIAL TEMPERATURE AS A BIOMARKER FOR HEPATOCELLULAR CARCINOMA

El-Badri, Nagwa - *Biomedical Science, Zewail City of Science and Technology, October City, Egypt*
Gaser, Ola - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Nasr, Mohamed - *Biomedical science, University of North Carolina, North Carolina, NC, USA*
Hussein, Alaa - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Ayman, Radwa - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Saad, Shams - *Biological science, Purdue Univeristy, West Lafayette, IN, USA*
AbdElmourady, Nourhan - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Elmehrath, Ahmed - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Ehab, Seif - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*

Abstract: Previous research showed that the mitochondria function at high temperatures that can reach 50°C.. These unique bioenergetic functions were attributed to the action of electron transport chain enzymes that carry on oxidative phosphorylation. In hepatocellular carcinoma (HCC), oxidative phosphorylation could be slowed down or shut as a result of shifting to phenomenon known as the Warburg effect. We hypothesized that HCC exhibits lower mitochondrial temperature due to metabolic switching from oxidative phosphorylation to glycolysis. We used HEPG2 HCC cell line treated it with Metformin to shift its metabolism and induce glycolysis. We used Mito thermo yellow (MTY) stain to measure the mitochondrial temperature under physiological conditions. After inducing hypoxia into HepG2 cells, we evaluated the glycolytic induction genotypically using qRTPCR. The molecular mechanism of heating in cancer cells was assessed by measuring the produced ATP, NADPH and ROS. Finally, the mitochondrial dynamics were assed via transmission electron microscopy (TEM). Upon Metformin treatment, HepG2 HCC cells showed a decrease in MTY fluorescence intensity, Glycolytic genes were up-regulated by 3-60 folds compared to only 2 folds in oxidative phosphorylation genes. The molecular mechanism of heating showed up regulation in both ATP and ROS production, but a decrease in NADPH production in Metformin treated cells. TEM showed a higher mitochondrial fragmentation but a lower mitochondrial count in Metformin treated cells compared to non-treated ones. These data support the hypothesis of a lower mitochondrial temperature as a result of induced glycolysis. Further research is required unravel the underlying mechanisms of mitochondrial cooling in cancer cells.

Funding Source: This work is supported by grant ASRT Jesor #5275, funded by The Academy of Scientific Research and Technology (ASRT), Egypt to NE-B and Zewail City of Science and Technology internal fund (2019-003).
Keywords: Mitochondrial temperature, Hepatocellular carcinoma, Mitochondrial bioenergetics

POSTER SESSION I: EVEN

7:30 PM – 8:30 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: CARDIAC

502

ENGINEERING OF SPECIFIC MICRORNA-DEPLETED PROGENITOR CELL-DERIVED SMALL EXTRACELLULAR VESICLES FOR MYOCARDIAL INFARCTION TREATMENT

Park, Hyun-Ji - *Biomedical Engineering, Emory University, Atlanta, GA, USA*
Hoffman, Jessica - *Graduate Division of Biological & Biomedical Sciences, Emory University, Atlanta, GA, USA*
Brown, Milton - *Biomedical Engineering, Emory University, Atlanta, GA, USA*
Davis, Michael - *Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA*

Abstract: Stem and progenitor cells are rapidly emerging therapeutic sources that have already demonstrated a lot of potential in clinical trials. Recently, emerging evidence have demonstrated that stem and progenitor cell therapy functions via paracrine signaling, not via differentiation and replacement of the infarcted tissue. The paracrine signaling utilizes extracellular vesicles (EVs) to deliver molecular cargo between cells or tissue, which have been identified to have a critical role in cardiac cell therapy. Among sEV cargo molecule types, microRNA (miRNA) is particularly potent but highly heterogenous. Thus, in this study, we identified deleterious small EV (sEV) miRNAs from our previously published computational models and knock down these miRNAs to develop engineered sEVs. Our engineered sEVs exhibited enhanced therapeutic capabilities in vitro and in a rat model of cardiac ischemia reperfusion. Specifically, our computational partial least square regression models revealed two deleterious sEV cargo miRNAs, miR-192-5p and miR-432-5p, which covary with reduced left ventricular ejection fraction (LVEF), anti-angiogenic, and pro-fibrotic responses in vitro and in vivo. Therefore, we investigated the effect of cardiac c-kit+ progenitor cell (CPC)-derived sEVs with miR-192-5p or miR-432-5p knockdown in treatment of chronic myocardial infarction model. We treated miR-192-5p and miR-432-5p knocked down CPC-sEVs to activated fibroblasts, inflamed monocytes, and mesenchymal stromal cell (MSCs), as well as directly injected sEVs to infarcted myocardium in chronic rat model of cardiac ischemia-reperfusion. The result showed that our miR-192-5p and miR-432-5p depleted engineered CPC-sEVs enhanced cardiac function by reducing fibrosis, enhancing mesenchymal stromal cell-like cell mobilization, and inducing macrophage polarization to the M2 phenotype. Also, engineered CPC-sEV injection to ischemic myocardium improved LVEF and fractional shortening, as well as reduced fibrosis and hypertrophy in left ventricle. Considering no treatment exists to fundamentally address chronic MI, engineered CPC-sEVs provide a cell-free system that leverages the therapeutic potential of CPCs while avoiding the risks associated allogeneic cell transplantation.

Funding Source: This study was supported by grants from National Institute of Health (R01HL145644 to M.E.D.; F31HL154725 and T32GM008602 to J.R.H) and the American Heart Association (AHA) Postdoctoral Fellowship (837187 to H.-J.P.).

Keywords: Cardiac progenitor cell, Small extracellular vesicles, microRNA

TOPIC: EPITHELIAL_GUT

504

INTESTINAL ORGANOIDS FOR AUTOMATED SCREENING ASSAYS. HIGH CONTENT IMAGING AND ANALYSIS OF ORGANOID MORPHOLOGY

Sirenko, Oksana - *Research and Development, Molecular Devices, San Jose, CA, USA*
Brandenberg, Nathalie - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Clapés, Maria - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Lim, Angeline - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*
Macha, Krishna - *Marketing, Molecular Devices, LLC, San Jose, CA, USA*
Meyer, Marine - *Research and Development, SUN bioscience, Lausanne, Switzerland*

Abstract: 3D cell models representing various tissues were successfully used for studying complex biological effects and tissue architecture; however, complexity of 3D models remains a hurdle for the wider adoption in research and drug screening. We describe the automated integrated cell culture and a high-content imaging system that allows automated monitoring, maintenance, characterization of organoids and testing the effects of various compounds. The integrated system included confocal imaging system, automated incubator, liquid handler as well as collaborative robot. We developed methods for automation of the seeding, media exchange, as well as monitoring development of mouse intestinal organoids. In addition, this method allows automation of compound testing and evaluation of toxicity effects. 3D intestinal organoids were developed from mouse intestinal cells cultured in matrigel. Using automated liquid handling system allowed automated seeding cell in Matrigel droplets followed by automated media addition and media exchanges. Organoids were monitored using imaging in transmitted light. Then machine learning-based image analysis allowed detection of organoids and characterization of their size and density. For endpoint measurements organoids were stained with fluorescently labeled antibodies or viability dyes and imaged using the automated confocal imaging system. Advanced image analysis allowed by 3D reconstitution and complex phenotypic evaluation of organoid structures, including characterization of organoid size and complexity, cell morphology and viability, and presence of differentiation markers. We demonstrated concentration-dependent toxicity effects of several anti-cancer drugs. We further increased the complexity by generating human intestinal organoids in Gri3D micropatterned U-bottom shaped micro-wells in hydrogel. We followed the development and self-organization of healthy human intestinal organoids over time which allowed the assessment of phenotypic features at a single-organoid level in an automatable high throughput workflow. We demonstrate the tools for increasing throughput and automation of organoid assays and compound screening, and also propose analysis approaches and descriptors that allow to gain more information about these complex models.

Keywords: intestinal organoids, automation, high content imaging

TOPIC: HEMATOPOIETIC SYSTEM

506

LOW-COST FLUOROSILANE-MODIFIED FILTROPORATION DEVICES ENABLING GENE KNOCKOUT IN HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Frost, Isaura M. - *Bioengineering, University of California, Los Angeles, Marina Del Rey, CA, USA*
Mendoza, Alexandra - *Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA*
Chiou, Tzu-Ting - *Pediatrics, UCLA, Los Angeles, CA, USA*
Kim, Philseok - *SLIPS Manufacturing, Adaptive Surface Technologies, Inc., Cambridge, MA, USA*
Aizenberg, Joanna - *Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA*
Kohn, Donald - *Molecular and Medical Pharmacology, UCLA, Los Angeles, CA, USA*
De Oliveira, Satiro - *Pediatrics, UCLA, Los Angeles, CA, USA*
Weiss, Paul - *Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA*
Jonas, Steven - *Pediatrics, UCLA, Los Angeles, CA, USA*

Abstract: Monogenic hematologic disorders that present in childhood are challenging therapeutic targets for which definitive treatment options remain limited. Over 400,000 children (predominantly in Africa and South Asia) are born each year with a β-hemoglobinopathy. Delivery of biomolecular cargoes that are required for gene manipulations to enable basic science investigations and/or manufacturing of targeted gene therapies, such as CRISPR/Cas9, is non-trivial and current commercial intracellular delivery technologies fall short due to toxicities, low throughput/efficiency, high costs, and the requirement for specialized equipment. Electroporation or nucleofection, which porate cells by application of electric charges, have been shown to cause transcriptional anomalies in stem cell populations. To circumvent these issues, novel techniques have been developed that employ mechanical cell deformation to permeabilize cells. Our group and others have shown that a variety of biomolecules can be efficiently delivered to immortalized and primary cells in a minimally toxic way via this approach. However, the requirement for specialized equipment in the form of custom microfluidic chips and fluid handling systems has, to date, limited the broader adoption of these technologies. Here, we report a device for intracellular delivery that can be assembled from materials easily accessible and often readily available in research laboratories. Our filtroporation devices employ a simple porous cell culture insert to deform target cells. Cells are rendered permeable as they are pulled through the insert's pores by application of vacuum available in biosafety cabinets. In a format that costs <$10 in materials per experiment, we demonstrate delivery of dextrans and Cas9 ribonucleoproteins for gene knockout to human CD34+ hematopoietic stem and progenitor cells (HSPCs) with knockout efficiencies >20% and minimal toxicities. When comparing gene knockout efficiency per micromolar concentration of cargo used in solution, our device outperforms nucleofection. These table-top approaches offer a versatile and straightforward solution to democratize access to transfection technologies for genetic manipulations, thus enabling more laboratories from low-resource areas of the world to engage in basic stem cell research.

Funding Source: Alex's Lemonade Stand Foundation NIH Director's Early Independence Award (DP5)

Keywords: Intracellular Delivery, Hematopoietic Stem Cells, Gene knockout

TOPIC: IMMUNE SYSTEM

508

EVALUATION OF ORTHOGONAL ANALYSIS METHODS TO QUANTIFY VECTOR COPY NUMBER

Kandell, Jennifer - *Pharma Services Group, Thermo Fisher Scientific, La Jolla, CA, USA*
Milian, Steven - *Pharma Services Group, Thermo Fisher Scientific, Alachua, FL, USA*
Snyder, Richard - *Pharma Services Group, Thermo Fisher Scientific, Alachua, FL, USA*
Lakshmipathy, Uma - *Pharma Services Group, Thermo Fisher Scientific, San Diego, CA, USA*

Abstract: Robust analytical methods with high sensitivity and specificity are required to characterize ex vivo modified cells and satisfy current regulatory expectations. The determination of vector copy number for lenti-modified cells is a crucial assay to distinguish safety in addition to identity, purity and potency assays. Due to factors including the presence of interfering components in cell samples and during processing steps, accurate determination of copies of the gene integrated into the host cell genome can be challenging. Relative quantification using quantitative Real-time PCR (qPCR) is a commonly used method that relies on serial dilutions of a standard harboring the target gene. Similarly, droplet digital PCR (ddPCR) is an orthogonal method that performs absolute quantification based on the principles of sample partitioning and Poisson distribution. This study evaluates vector copy number in lentivirus modified cells based on a detailed evaluation of qPCR and ddPCR methods. Analytical assay sensitivity and performance were assessed using standards and controls to achieve a range of target copies. Additionally, lower limit of quantification (LLOQ) was established for both methods. Matrix effects on assay performance in addition to inter- and intra- assay variability were measured. Overall, correlation of the results from qPCR and ddPCR methods was tested using a linear regression model. This detailed approach to method development based on ICH guidelines enables easier assay transfer from research to translational purposes for further qualification in GMP.

Keywords: Vector Copy Number, qPCR, ddPCR

TOPIC: MUSCULOSKELETAL

510

ENGINEERING MULTIPENNATE 3D SKELETAL MUSCLE TISSUE USING FRESH 3D BIOPRINTING

Stang, Maria A. - *Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*
Lee, Andrew - *Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*
Coffin, Brian - *Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*
Yerneni, Sai - *Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*
Bliley, Jaci - *Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*

Campbell, Phil - *Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*
Feinberg, Adam - *Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA*

Abstract: Muscle disease and injury affects millions of individuals each year and can lead to significant impairment or disability. Functional free muscle transfer is the clinical standard for repair but results in donor site morbidity and often is not viable due to the size and complexity of the muscle defect. Engineered skeletal muscle tissue is a potential solution, but current tissue engineering approaches cannot recreate the complex, 3D anisotropic architectures of many pennate muscle types. Here, we report the engineering of skeletal muscle tissues with highly aligned parallel, unipennate, bipennate, and multipennate myofiber architectures. To do this, we used Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting of collagen type I scaffolds that matched these muscle types. Next, we cellularized these scaffolds with murine myoblast progenitor C2C12 cells at a concentration of 30 million cells/mL by casting them in a collagen solution around the scaffolds and using cell-mediated compaction to drive cell infiltration. Scaffolds were designed with 100 um diameter collagen filaments and defined spacing optimized to maximize cell infiltration, alignment to the scaffold, and fusion and differentiation into contractile myotubes. Confocal imaging confirmed scaffold fidelity and that the cells organized and aligned along the collagen filaments and fused into multinucleated myotubes. Under field stimulation, engineered muscle tissue displayed synchronized contraction, calcium transients, and a positive force-frequency relationship. Tissues were also subcutaneously implanted into C3H mice for ten days. Histology showed that scaffold architecture was maintained for 10 days and in select areas guided anisotropic vascular ingrowth parallel to the differentiated myotubes. Together, these results demonstrate that collagen can be FRESH 3D bioprinted in various skeletal muscle architectures with high fidelity to guide tissue organization, generate contractions with a positive force-frequency relationship, and direct vascularization in vivo.

Keywords: muscle progenitor cells, 3D bioprinting, skeletal muscle architectures

TOPIC: NEURAL

512

MACHINE LEARNING DRIVEN TSC DISEASE REVERSION TESTING IN AN IPSC DERIVED CORTICAL NEURON DISEASE MODEL

Tu, Shengjiang - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Salick, Max - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Sivanandan, Srinivasan - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Marie-Nelly, Herve - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Velayutham, Jeevaa - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Mohammadi, Shahin - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Dreossi, Tommaso - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Atmaramani, Rahul - *Neuroscience, Insitro, South San Francisco, CA, USA*
Pan, Meiliang - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Yi, Flora - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Sharon, Elion - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Chu, Ci - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Kaykas, Ajamete - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*

Abstract: Tuberous sclerosis complex (TSC) is a multisystem genetic disorder, with high disease penetrance in the neurological system, highlighted by epilepsy in more than 80% of patients. TSC is caused by mutations in TSC1 or TSC2 which lead to mTORC1 pathway hyperactivity. Current treatment options are limited. Here in an attempt to build a predictive in vitro disease model, we introduced TSC2 genetic knockout (KO) into human iPSC derived cortical neurons, collected multi-modal and longitudinal molecular and imaging data (biomarker, bulk and scRNAseq, fluorescent and live cell imaging), and built machine learning (ML) phenotypic models that can describe the biological states of the sick neurons and the healthy isogenic controls. We showed that the ML phenotypic disease models can be used for target / drug discovery by demonstrating time and dose dependent phenotypic reversion using a mTOR inhibitor. The phenotypic reversion is highly concordant across orthogonal data modalities. Lastly, we verified the relevance of in vitro ML disease models to in vivo pathology by correctly distinguishing transcriptomic datasets generated from subependymal giant cell astrocytoma extracted from TSC patients vs. periventricular control tissues. Thus, we demonstrated a rapid approach to engineering predictive iPSC based disease models that can be leveraged for multi-modal, ML-driven phenotypic drug discovery.

Keywords: TSC, cortical neurons, Machine learning

TOPIC: NT - GENERAL

516

INCREASING HUMAN MSC YIELDS THROUGH UNBROKEN PHYSIOXIA

Henn, Alicia - *Scientific, BioSpherix, Parish, NY, USA*
Darou, Shannon - *Scientific, BioSpherix, Parish, NY, USA*
Yerden, Randy - *Scientific, BioSpherix, Parish, NY, USA*

Abstract: There is a large body of literature that documents the stresses that supraphysioxic conditions place on human bone marrow mesenchymal stromal/stem cells (MSC), yet traditional room air culture practices with these clinically relevant cells still persist. We tested the hypothesis that MSC provided with physioxia (3 – 5% O2) just for incubation, and then handled in the supraphysioxia of room air (as in a biological safety cabinet), would have equivalent growth characteristics as MSC maintained in unbroken physioxia and CO2. Human bone marrow MSC cultured in triplicate T-75 flasks of MSC were housed for up to 8 passages in a traditional room air incubator fitted with an oxygen-controlled subchambers set to 5% O2/5% CO2. They were placed in HEPA-filtered room air conditions (Room-Air) for cell handling. The other set of cultures were housed within a closed processing chamber (Xvivo System) for full-time control of oxygen and CO2. Cell culture media were pre-equilibrated to the matching cell handling conditions. Cell growth was recorded using the CytoSMART

cell imaging system. We found that the two sets of conditions did not produce equivalent cell growth. There were higher yields in each passage, and more total cell passages in cultures maintained full-time in physioxic conditions than in cultures handled under traditional supraphysioxic room air conditions (two-tailed T test, unequal variances). We went on to look at how long it took pericellular and intracellular oxygen levels to recover after a quick room air medium change with medium that was not pre-equilibrated to physioxic levels. We measured pericellular oxygen levels with an oxygen probe and intracellular oxygen with an intracellular oxygen indicator dye. Pericellular oxygen levels in cultures handled in room air took over 80 minutes to equilibrate to the physioxia, far longer than it takes to modulate HIF-1a levels in MSC. Intracellular oxygen took even longer to recover, over 150 minutes. Therefore, controlling oxygen levels around MSC during cell handling operations is critical to maximizing MSC yields both for research and clinical applications.

Keywords: MSC, oxygen, mesenchymal stromal cells

518

ENHANCED ISOLATION OF HIGH-QUALITY HUMAN MESENCHYMAL STROMAL CELLS UNDER XENO-/SERUM-FREE CONDITIONS

Thamm, Kristina - *Project Services, denovoMATRIX, Dresden, Germany*
Möbus, Kristin - *Medizinische Klinik und Poliklinik 1, Universitätskrankenhaus Carl Gustav Carus der Technischen Universität Dresden, Germany*
Towers, Russel - *Medizinische Klinik und Poliklinik 1, Universitätskrankenhaus Carl Gustav Carus der Technischen Universität Dresden, Germany*
Baertschi, Stefan - *CELLnTEC, CELLnTEC advanced cell systems AG, Bern, Switzerland*
Wetzel, Richard - *denovoMATRIX, Dresden, Germany*
Wobus, Manja - *Medizinische Klinik und Poliklinik 1, Universitätskrankenhaus Carl Gustav Carus der Technischen Universität Dresden, Germany*
Segeletz, Sandra - *Innovation and Reserach, denovoMATRIX, Dresden, Germany*

Abstract: The systematic application of cell-based therapies requires effective cell manufacturing approaches determined by the efficiency of cell isolation from donor tissue and subsequent in vitro expansion. Mesenchymal stromal cells (MSCs) are a frequently used resource for treating complex pathologies. However, their isolation is constrained by the low abundance of MSCs in the tissues of origin and by donor-specific characteristics, including MSC frequency/quality that decline with disease state and increasing age. Thus, optimizing the isolation efficiency would significantly improve the final cell yield of a manufacturing process and is essential to serve the acute need for therapeutically active MSCs. We, therefore, developed a chemically defined biomimetic surface coating (isoMATRIX) that facilitates the isolation of MSCs in xeno/serum-free and chemically defined conditions. The coating allows for MSC isolation with significantly higher cell yield and proliferation rate independent of the donor and tissue origin while maintaining robust immunomodulatory capacity. The impact of the isoMATRIX on cell manufacturing processes is highlighted in our model of an expansion process that implements the higher cell yield and faster cell proliferation. We calculated that using the isoMATRIX allows the treatment of twice as many patients or the same number of patients 3-7 times faster. Thus, the isoMATRIX presents an essential step towards routine cell therapies and is, to our knowledge, the first reported surface specifically designed for chemically defined MSC isolation.

Funding Source: denovoMATRIX GmbH was supported and received funding by the European Social Fund (ESF), the European Regional Development Fund (ERDF), and by the EXIST-Forschungstransfer granted by the BMWi.
Keywords: mesenchymal stromal cells, isolation, biomatrices

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A UNIVERSAL DNA READ INTERPRETER FOR SINGLE-CELL AND SPATIAL GENOMICS

Kijima, Yusuke - *School of Biomedical Engineering, Faculty of Applied Science and Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*
Yamamoto, Daniel - *Synthetic Biology Division, Research Center for Advanced Science and Technology, The University of Tokyo, Setagaya, Japan*
Toyoshima, Hiromi - *Synthetic Biology Division, Research Center for Advanced Science and Technology, The University of Tokyo, Setagaya, Japan*
Yachie, Nozomu - *School of Biomedical Engineering, Faculty of Applied Science and Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada*

Abstract: The concept of DNA barcoding with high-throughput sequencing has triggered the recent rapid growth of single-cell and spatial genomics technologies. The new technologies have enabled the high-content characterization of cell statuses, tissue structures, and their dynamics and underpinning molecular mechanisms. However, the engineering cost of data analysis pipelines for the development and modification of such technologies remains high—each technology produces uniquely structured sequencing libraries and often requires the development of a specific data analysis pipeline to decode their highly-contexed read structures. In this conference, we will report a universal platform INTERSTELLAR (interpretation, scalable transformation, and emulation of large-scale sequencing reads) that extracts data values encoded in theoretically any type of sequencing reads and translates them into those of another structure. We demonstrated that INTERSTELLAR successfully extracted information from various complex sequencing libraries for downstream data analyses and emulated those of single-cell (sc)RNA-seq, scATAC-seq, and spatial transcriptome sequencing each to be analyzed by different software tools that have been developed for similar types of experiments. INTERSTELLAR accelerates the development of new sequencing-based experiments with a minimal coding effort by effectively synergizing the codes developed for a range of sequencing-based genomics technologies.

Keywords: DNA barcode, Single-cell omics, Spatial transcriptomics

TOPIC: PLURIPOTENT STEM CELLS

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OPTIMISED CRISPR-CAS9 MEDIATED AND BASE EDITING OF IPSCS FOR DISEASE MODELLING

Cuevas Ocana, Sara - *Nottingham Biodiscovery Institute, University of Nottingham, UK*
Serna-Valverde, Ana-Lilia - *Nottingham Biodiscovery Institute, University of Nottingham, UK*

Reed, Liam - *Nottingham Biodiscovery Institute, University of Nottingham, UK*
Hannan, Nicholas - *Nottingham Biodiscovery Institute, University of Nottingham, UK*

Abstract: Patient-derived induced-pluripotent stem cells (iPSCs) have been shown to be powerful tools for disease modelling and patient-specific drug testing. Genetic engineering technologies have played a key role in the development of genetically corrected healthy counterparts for patient-derived iPSCs and de novo introduced modifications for disease modelling. Zing Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 mediated iPSC gene-editing have shown success, but efficiency remains an important challenge. Based on our previously developed method for TALEN and CRISPR-Cas9 mediated gene-editing of iPSCs achieving correctly modified cells in ~ 3 weeks with 1-10% efficiency, we have explored further conditions to increase iPSC gene-editing efficiency. Here, we describe the optimisation of transfection, guideRNA/nuclease ratio, screening strategies at population level, clonal isolation and screening for CRISPR-Cas9 mediated iPSC gene-editing. We also performed in parallel comparisons of the gene-editing efficiency obtained with our optimised CRISPR-Cas9 mediated method and the recently reported base editing systems. Cytidine and adenine base editing enable the conversion of a single base to another (C:G-to-T:A and T:A-to-C:G) without the need to introduce DNA double-strand breaks. Using idiopathic pulmonary fibrosis (IPF) patient-derived iPSCs carrying a heterozygous mutation, we have demonstrated that base editing systems overperformed our optimised CRISPR-Cas9 mediated gene-editing results, achieving over 80% gene-editing efficiency and requiring fewer clonal isolation events. Furthermore, we confirmed that the iPSCs modified by base editors maintain a normal karyotype, pluripotency marker expression and differentiation potential. Using our in-house developed lung differentiation protocol, we showed that iPSCs modified by cytidine and adenine base editing can generate alveolar type 2 (AT2) cells, highly relevant for the study of IPF in a patient-specific manner. Our optimised conditions for standard CRISPR-Cas9 mediated and base-editing methods will advance application of iPSC-based disease modelling and improve the clinical suitability of gene-edited hIPSCs.

Keywords: Gene editing, Pluripotent stem cells, Disease modelling

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ILLUMINATING CELLULAR IRON HOMEOSTASIS IN EARLY CELL FATE DECISIONS AT SINGLE-CELL RESOLUTION

Sangokoya, Carolyn - *Pathology, University of California, CA, USA*
Blelloch, Robert - *Urology, University of California, San Francisco, CA, USA*

Abstract: Post-transcriptional regulation by RNA-binding proteins orchestrates diverse cellular mechanisms that regulate pluripotent stem cell biology and pattern early mammalian development. Iron regulatory (IRP) RNA-binding proteins are important for early mammalian embryonic development, as their complete loss leads to lethality and partial loss leads to developmental defects and morbidity. However while it is well known that iron is essential for life at the molecular, cellular, and systemic/organismal levels, the distinct roles for cellular iron regulation in pluripotent stem cells as they undergo pivotal state transitions and early cell fate decisions are not well characterized. Our previous studies defined an axis of microRNA-dependent post-transcriptional control and endocytosis that is critical for signal transduction of the fibroblast growth factor-extracellular signal-regulated kinase (FGF-ERK) signaling pathway in pluripotent cell state transition and early differentiation. Our most recent findings further reveal that coordinate control of this axis by IRP RNA-binding proteins fine-tunes lineage commitment. By introducing new methods for the analysis of IRP RNA-binding activity and cellular iron levels at single-cell resolution, we reveal that dynamic post-transcriptional regulation controls both intracellular iron levels and cell fate markers during trilineage specification using stem-cell based gastrulation-stage embryo models. Our initial findings shed light on post-transcriptional regulation by RNA-binding proteins as a dynamic mechanism capable of driving metabolic crosstalk and fine-tuning cell fate.

Funding Source: NIH/Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant K08HD105017
Keywords: cell fate, RNA-binding proteins, iron

526

A CLOSED-SYSTEM SOLUTION DESIGNED TO GENTLY AND RAPIDLY PROCESS PSC SPHEROIDS FROM BIOREACTORS

Akenhead, Michael L. - *Research and Development, Thermo Fisher Scientific, Frederick, MD, USA*
Kennedy, Mark - *Research and Development, Thermo Fisher Scientific, Frederick, MD, USA*
Kuninger, David - *Research and Development, Thermo Fisher Scientific, Frederick, MD, USA*

Abstract: Large-scale growth of pluripotent stem cells (PSCs) is a bottleneck for many therapeutic and screening applications requiring substantial cell quantities. This challenge can be overcome using bioreactors to grow three-dimensional (3D) PSC spheroid suspension cultures. However, processing the yields generated in a closed-system environment is often difficult. Therefore, we sought to alleviate this issue using the Cell Therapy Systems (CTS) Rotea Counterflow Centrifugation system. Here, we describe how to transfer spheroids grown in StemScale PSC Suspension Medium from a bioreactor into the Rotea, dissociate the spheroids into single cells, and harvest the cell suspension, all while maintaining a closed system environment. With optimized centrifugation speed, the 3L bioreactor spheroid yield can gently flow through the Rotea tubing with minimal shear stress in under 40 minutes. Once loaded, spheroids can be rapidly dissociated in under 10 minutes, 3-4 times faster than water bath protocols. The resulting cell yield was seeded into new suspension cultures, enabling growth of pluripotent spheroids in subsequent passages and their use in downstream differentiation. Overall, this optimized protocol demonstrates the in-line use of bioreactors and the Rotea system to be a convenient method to efficiently process large amounts of PSC spheroids in a therapeutic-friendly, closed system environment.

Keywords: spheroid, bioreactor, suspension culture

TOPIC: NT - GENERAL

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USING CANINE ORGANOIDS TO ADVANCE THERAPEUTIC DRUG DEVELOPMENT IN BLADDER CANCER: A ONE HEALTH APPROACH

Wickham, Hannah M. - *Ankeny, IA, USA*
Mosichuk, Allison - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Sahoo, Dipak - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Zdyrski, Christopher - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Gabriel, Vojtech - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Wickham, Hannah - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Iennarella-Servantez, Chelsea - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Bourgois-Mochel, Agnes - *Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA*
Trepanier, Lauren - *Department of Medical Sciences, University of Wisconsin-Madison, WI, USA*
Thomson, Chris - *Veterinary Specialty Hospital, Ethos Veterinary Health, San Marcos, CA, USA*
Lucien, Fabrice - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Frank, Igor - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Cheville, John - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Allenspach, Karin - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*

Abstract: Up to 50% of patients with muscle-invasive bladder cancer (MIBC) fail to respond to standard of care platinum-based neoadjuvant chemotherapy (NAC e.g., cisplatin); a disease associated with a survival rate of only 40% at five years follow-up. Two important factors that contribute to poor treatment outcomes are (1) the lack of truly innovative and effective drug candidates for MIBC, and (2) the phenotypic and molecular heterogeneity of MIBC tumors which hampers the translational value of most rodent models. Dogs with naturally occurring MIBC constitute an ideal preclinical study population to assess novel therapeutic options due to similarities in genetic predispositions, environmental risk factors, tumor molecular phenotypes, clinical etiology and treatment response. The further development of novel assays for the prediction of chemotherapeutic efficacy before in vivo testing in dogs with MIBC can streamline and accelerate drug development timelines. A promising technology in this regard consists in the culture of 3D patient-derived tumor organoids (PDOs). The aim of this preliminary study was to demonstrate proof-of-feasibility that canine organoids can be cultured from free catch urine, recapitulate key molecular features of their original tumors, and be used for functional cytotoxicity assays with NAC. Briefly, 3D organoids from MIBC canine patients were obtained using a modified version of our standard protocol for growth of canine intestinal organoids. Findings from RNA in situ hybridization showed a high degree of redundancy in marker expression between canine PDOs and their parent tumors for CK7 and CD44, two markers associated with urothelial carcinoma and poor prognosis in human patients with MIBC, respectively. CellTiter-Glo 3D cell viability assays (Promega) with cisplatin at increasing concentrations confirmed functional cytotoxicity in 3D organoids with an estimated IC50 of 0.28 (±0.03) μM. These preliminary results suggest that urinary-derived canine MIBC organoids share molecular features with their tumor of origin and are metabolically active. Collectively, these findings show the potential value of 3D organoids to predict therapeutic response in bladder cancer prior to in vivo testing in canine and human patients with MIBC.

Funding Source: Barry Cancer Research Foundation
Keywords: Bladder Cancer, 3D Organoids, One Health

TOPIC: EARLY EMBRYO

752

N6-METHYLADENOSINE REGULATES MATERNAL RNA MAINTENANCE AND TIMELY ZGA RNA DECAY DURING MOUSE MZT PROGRESSION

Wu, You - *School of Life Sciences and Technology, Tongji University, Shanghai, China*
Xu, Xiaocui - *School of life and technology, Tongji University, Shanghai, China*
Qi, Meijie - *State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China*
Shen, Bin - *State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China*
Gao, Yawei - *School of Life Sciences and Technology, Tongji University, Shanghai, China*
Gao, Shaorong - *School of Life Sciences and Technology, Tongji University, Shanghai, China*

Abstract: N6-methyladenosine (m6A) and its regulatory components play critical roles in various developmental processes in mammals. However, the landscape and function of m6A in early embryos remain unclear due to limited materials. We developed an ultra-low-input MeRIP-seq method and found unique enrichment and dynamics of m6A RNA methylomes on maternal and zygotic RNAs including the transposable elements MTA and MERVL. Notably, we found that the maternal protein KIAA1429, a component of the m6A methyltransferase complex, was essential for m6A on zygotic decay (Z-decay) maternal mRNAs and MTA to stabilize their high abundance in oocytes. Interestingly, m6A methyltransferases, especially METTL3, regulated the establishment of m6A on zygotic genome activation (ZGA) mRNAs and ensured the decay of 2-cell-specific mRNAs, including Zscan4 and MERVL. Together, our findings uncover the essential functions of m6A in specific contexts during the maternal-to-zygotic transition (MZT), namely, ensuring transcriptome stability in oocytes and regulating the stage specificity of zygotic transcripts after fertilization.

Keywords: m6A, early embryo, retrotransposon

TOPIC: IMMUNE SYSTEM

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UNDERSTANDING THE IMMUNOGENICITY OF HUMAN PLURIPOTENT STEM CELLS VIA AN IMMUNOPEPTIDOMICS APPROACH

Huang, Ziyi - *Biochemistry and Molecular Biology, Monash University, Clayton, Australia*
Laslett, Andrew - *CSIRO Manufacturing, CSIRO, Melbourne, Australia*
Purcell, Anthony - *Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia*
Illing, Patricia - *Monash University, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Melbourne, Australia*

Abstract: It has been a century since the first report showed that vaccination of animals with embryonic tissues could prevent the outgrowth of tumours. Subsequent studies have confirmed that immune cells, in particular T cells, can be primed against multiple types of tumour by embryonic tissues and pluripotent stem cells (PSCs). The rationale for using PSCs is based on the overlap in transcriptome profiles between these cells and tumours. Moreover, current evidence suggests PSCs may display similar antigens to those presented by cancer stem cells/cancer-initiating cells. Hence, PSC-based vaccines are proposed to aid in both the prevention of tumour initiation and tumour recurrence. However, the immunogenicity of PSCs remains controversial and the precise antigens that contribute to their immunogenicity are unknown. Therefore, we have initiated a mass spectrometry-based epitope discovery program, interrogating the HLA-I immunopeptidome of human PSCs to define the mechanisms that enable PSCs to stimulate anti-cancer immune responses. Current work moves to explore the naturally presented HLA-I immunopeptidome of PSCs to determine similarities and differences with cancerous and healthy human tissues. These studies will help define the features of PSCs that stimulate anti-cancer responses, informing the design of cancer vaccines.

Keywords: Immunopeptidomics, Major histocompatibility complex (MHC), Cancer

TOPIC: NEURAL

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DIRECTED DIFFERENTIATION OF DOPAMINERGIC NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS THROUGH MICROFLUIDIC CELL SQUEEZE® DELIVERY OF MULTIPLE MRNA ENCODING TRANSCRIPTION FACTORS

Wu, Chun-I - *Exploratory, SQZ Biotechnologies, Watertown, MA, USA*
Ozkan, Abdulkadir - *Exploratory, SQZ Biotech, Watertown, MA, USA*
Silva, Murillo - *Exploratory, SQZ Biotech, Watertown, MA, USA*
Gilbert, Jonathan - *Exploratory, SQZ Biotech, Watertown, MA, USA*
Bridgen, Devin - *Exploratory, SQZ Biotech, Watertown, MA, USA*

Abstract: Generating therapeutic cells to replace lost or diseased cells is a promising approach for currently intractable diseases such as Parkinson's disease. One attractive cell source for cell replacement therapies is induced pluripotent stem cells (iPSCs) since they can differentiate into a wide variety of somatic cells. However, the differentiation process through the sequential activation of key signaling pathways with small molecules is a lengthy and variable process. More recently, the forced expression of a key set of lineage-specifying transcription factors enabled cell differentiation with higher efficiency, homogeneity, and speed but typically requires the use of viral or integrating vectors, which pose safety concerns for clinical use. Cell Squeeze® technology enables non-viral, cytosolic delivery of a variety of materials while preserving cell health and limiting adverse effects on baseline gene expression. Furthermore, our technology allows us to control the timing, intensity, and combination of transcription factor expression to create high-quality and functional cell products. In a new application of the Cell Squeeze® technology, we demonstrated the generation of dopaminergic neurons by simultaneously delivering six mRNAs encoding transcription factors into iPSCs with a single step. qPCR characterization of the induced neurons showed a >600x increase in dopaminergic neuron specification marker FoxA2 after only 4 days demonstrating the rapid initiation of endogenous transcriptional cascades. Immunofluorescence staining demonstrated the robust expression of a key dopaminergic neuron marker tyrosine hydroxylase and a mature neuronal marker MAP2 after 14 days. This work demonstrated the potential of the Cell Squeeze® technology to simultaneously deliver multiple transcription factors in synthetic mRNA form to drive the differentiation of clinically relevant cell types, including dopaminergic neurons for Parkinson's Disease, which has implications for a wide variety of regenerative medicine applications.

Keywords: Cell replacement therapy, Dopaminergic neurons, Intracellular delivery

TOPIC: NT - GENERAL

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ASSESSMENT OF TRANSCRIPTOMIC PROFILES OF PATIENT-DERIVED INTESTINAL ORGANOIDS BY BULK RNA-SEQUENCING

Boyd, Joseph S. - *Research and Development, MilliporeSigma, Temecula, CA, USA*
Chu, Vi - *Research and Development, MilliporeSigma, Temecula, CA, USA*

Abstract: Organoids are rapidly developing as important models for drug screening and useful cellular tools for recapitulation of in vivo physiology. In order to ascertain the relevancy of organoid cultures it is incumbent to characterize the transcriptomic profiles of organoid lines in their expanded, stem-cell enriched culture state. Six commercially-available patient-derived organoid lines consisting of normal colon and ileum and colon and colon rectum from Crohn's disease patients was subjected to high-throughput sequencing of bulk RNA. Published markers of colon and ileum were assessed and compared to a commercially-available iPSC-derived colonic organoid line. Differential gene expression analysis revealed patterns unique to Crohn's disease patients, in one line notably high differential expression of MUC5AC and MUC12, associated with colorectal cancer. Colon organoids from both Crohn's patients and normal colon tissue adjacent to carcinoma showed relative high expression of ileum marker genes.

Ongoing analysis of gene expression patterns in additional biobank samples will refine and clarify transcriptomic signatures of gastrointestinal samples that correlate with age, gender, and disease state.

Keywords: ORGANOIDS, TRANSCRIPTOMICS, CROHN'S DISEASE

TOPIC: PLURIPOTENT STEM CELLS

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MICROCARRIER CULTURE SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELLS USING LAMININ 511E8 FRAGMENT

Ebisu, Fumi - *Research and Development, Matrixome, Inc., Suita, Japan*
Minobe, Kohei - *Research and Development, Matrixome, Inc., Suita, Japan*
Sekiguchi, Kiyotoshi - *Institute for Protein Research, Osaka University, Suita, Japan*
Taniguchi, Yukimasa - *Institute for Protein Research, Osaka University, Suita, Japan*

Abstract: Human pluripotent stem cells (hPSCs) hold a great promise for regenerative medicine. However, a large number of cells are required for the clinical study, where conventional monolayer and 3D suspension culture systems might pose problem in scale-up and maintenance of critical cell functionality. Microcarrier culture system permits hPSC growth as monolayer on their surface area with the benefits of a homogeneous suspension culture. Nevertheless, this culture system with xeno-free matrix to support the strong hPSC adhesion and expansion has not been fully established yet. Recombinant laminin (LM) 511E8 fragment (e.g. = iMatrixTM-511) is a xeno-free truncated form of laminin-511 containing the binding site for the integrin α6β1 predominantly expressed on hPSCs. The strong interaction of LM511E8 with integrin α6β1 enables hPSCs to preserve pluripotency, maintain an undifferentiated state and sustain long-term single cell passaging. We aim to establish the microcarrier culture system in the human induced pluripotent stem cells (hiPSCs) with LM511E8. We first tested the coating efficiency of microcarriers with serially diluted LM511E8. The amount of LM511E8 coated on microcarriers increased in a dose-dependent manner in the range of 4 nM to 20 nM. We next screened the hiPSC adhesion and propagation on the LM511E8 coated microcarriers under adherent culture conditions. We observed cell attachment and expansion on microcarriers. Moreover, cells were positive for rBC2LCN, specifically expressed on hPSC surface. By referring to our results in 2D culture and previous work, we next investigated the stirred suspension culture for multiple-layer cell expansion on microcarriers with the working volume of a 5 mL bioreactor. We made a comparison of the amount of microcarriers, cell seeding density and stirring speed to acquire high proliferation. The maximum growth rate was obtained when we cultured hiPSCs with 5.6 mg of microcarriers and a seeding density of 6.0x105 cells stirring at 40 rpm. In addition, we confirmed the separation of hiPSCs from microcarriers with 5 mM EDTA/PBS(-). In conclusion, we established a series of workflows in a small-scale microcarrier culture system, which is easily scalable for therapeutic application.

Keywords: Microcarriers, Laminin 511E8, Human pluripotent stem cells

POSTER SESSION I: EVEN

7:30 PM – 8:30 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC:

602

GLOBAL CHANGES IN CHROMATIN ACCESSIBILITY DEFINE HIGHLY-EFFICIENT REPROGRAMMING

Galloway, Kate E. - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
Beitz, Adam - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
Johnstone, Christopher - *Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*

Abstract: High rates of proliferation mark highly-plastic cell populations in cancer and in cellular reprogramming. Yet, how proliferation globally impacts gene regulatory networks and epigenetic state remains poorly defined. Previously, we established that proliferation increases the reprogramming rate to a range of post-mitotic cells, indicating a transient role for proliferation in supporting the adoption of a new cell identify. As changes in accessibility often precede the adoption of a new fate, we sought to characterize changes in accessibility across populations of cell reprogramming from fibroblasts to post-mitotic motor neurons. Surprisingly, we find that hyperproliferative cells display globally reduced accessibility. In bulk ATAC-seq, we observe that hyperproliferative cells show a reduced fraction of transpositions in nucleosome-free regions, indicating a global reduction in chromatin accessibility. Similarly, via single-cell ATACseq, we find that hyperproliferative cell bear fewer transpositions per cell. Together these data suggest that high-efficiency reprogramming precedes through cells with globally reduced accessibility. Moreover, via single-cell RNAseq, we observe hyperproliferative cells traverse through an "undifferentiated" state characterized by limited expression of lineage-specific gene regulatory networks. Contrary to most models of reprogramming, our work suggests a hierarchy of processes in which globally reducing chromatin accessibility precedes activation of new gene regulatory networks. Our data suggest that proliferation-mediated de-differentiation represents a common motif in cell-fate transitions. As cell-division often precedes differentiation, proliferation may represent a common motif to support adoption of a new cell identity. While progenitor specific GRNs do not appear, the metabolic state of the highly plastic cells resembles the metabolic profile of neural progenitors. As cell-division often precedes differentiation, proliferation may represent a common motif to support adoption of a new cell identity. Potentially, the metabolic profile marked by rapid proliferation and high transcription rates, rather than the specific progenitor genes, confers plasticity and potentiates the adoption of new identities.

Funding Source: NIH NIGMS 1R35GM143033
Keywords: Mechanism of reprogramming, Cell-fate transition, Global changes in chromatin accessibility

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

604

MATRICELLULAR CYR61 IS ESSENTIAL FOR RETAINING THE PROPERTIES OF THE MESENCHYMAL STEM CELL NICHE IN AGING

Marinkovic, Milos - *Comprehensive Dentistry, University of Texas Health Science Center, San Antonio, TX, USA*
Dai, Qiuxia - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Gonzalez, Aaron - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Tran, Olivia - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Block, Travis - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Harris, Steven - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Yeh, Chih-Ko - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Dean, David - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*
Chen, Xiao-Dong - *Comprehensive Dentistry, University of Texas Health - San Antonio, TX, USA*

Abstract: Aging skeletal degeneration involves a decline in the quantity and quality of mesenchymal stem cells (MSCs). However, changes which occur in the ECM of the elderly bone marrow (BM) niche remain undefined. In this study, we produced ECMs ex vivo using BM stromal cells harvested from “young” (≤25 y/o) and “elderly” (≥60 y/o) donors and assessed their proteomic, mechanical and physical properties. We compared their ability to direct the responsiveness of cultured MSCs to osteogenic growth factors (BMP-2 & IGF-1). Relative to elderly extracellular matrix (eECM), young ECM (yECM) exhibited greater fibrillar organization and mechanical integrity; further, MSCs on yECM demonstrated significantly higher responsiveness to both BMP-2 and IGF-1. Proteomic analysis showed that Cyr61/CCN1, a matricellular protein containing binding motifs for these growth factors, was absent in eECM compared to yECM, and is generally associated with reduced YAP activation in elderly MSCs. Recently, others have shown that Cyr61 ablation in BM-MSCs significantly reduced bone mineral density (BMD) relative to wild type. However, these studies mainly focused on intrinsic (intracellular) Cyr61 expression, and its extrinsic role within the ECM remains unclear. To address this gap, we used genetic methods to increase or decrease incorporation of Cyr61 into ECM produced by elderly or young MSCs. Knock-down of Cyr61 in yECM abrogated responsiveness to both BMP-2 and IGF-1, indicating a significant role for ECM-bound Cyr61 in regulating osteogenesis. More importantly, replenishing Cyr61 content in eECM restored responsiveness of cultured MSCs to both growth factors. To investigate Cyr61 in the BM stromal niche in vivo, we compared BMD and Cyr61 content of L4-L5 vertebral bodies in “young” (9-11 m/o) and “elderly” (21-33 m/o) mice. These analyses demonstrated well-defined differences in BMD between the cohorts; further, tissues containing less BMD also exhibited a relative deficiency of Cyr61, based on WB and immunostaining of the vertebral bone matrix. Our study proposes a novel role for ECM-bound Cyr61 in directing MSC responsiveness to specific osteogenic growth factors, but also suggests depletion of Cyr61 from the BM stromal niche as a mechanism contributing to the dysregulation of MSCs in skeletal tissue aging.

Funding Source: VA Merit Review (1I01BX002145-01) NIH-NCATS TL1 Translational Science Training grant (TL1 TR001119) NIH-NIDCR F31 National Research Service Award (F31 DE02668).
Keywords: Aging Stem Cell Niche, ECM Proteomics, MSC Osteogenesis

TOPIC: EARLY EMBRYO

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BROAD MAINTENANCE OF PLURIPOTENCY IN DEVELOPING ECTODERM ENABLES NEURAL CREST STEMNESS AND CHALLENGES CURRENT VIEWS ON GASTRULATION

Kerosuo, Laura - *Neural Crest Development and Disease Unit, NIDCR / NIH, Rockville, MD, USA*
Pajanoja, Ceren - *NIDCR, NIH, Bethesda, MD, USA*
Hsin, Jenny - *NIDCR, NIH, Bethesda, MD, USA*
Yazeijan, Rita - *NDCR, NIH, Bethesda, MD, USA*
Olinger, Bradley - *NIDCR, NIH, Bethesda, MD, USA*
Takko, Heli - *Biochemistry and Developmental Biology, University of Helsinki, Finland*
Dapkunas, Arvydas - *Biochemistry and Developmental Biology, University of Helsinki, Finland*
Perera, Surangi - *NIDCR, NIH, Bethesda, MD, USA*
Zainul, Zarin - *NIDCR, NIH, Bethesda, MD, USA*
Doyle, Andrew - *NIDCR, NIH, Bethesda, MD, USA*
Schiffmacher, Andrew - *NIDCR, NIH, Bethesda, MD, USA*
Martin, Daniel - *NIDCR, NIH, Bethesda, MD, USA*

Abstract: Gastrulation irreversibly changes cells in the early embryo from pluripotent stem cells to restricted cells with potential to only form cell types specific for each germ layer. The neural crest rises from the ectoderm and doesn’t fit this rule by also forming mesodermal- and endodermal-like-cells, such as facial bone and cartilage, as well as chromaffin cells, in addition to the typical ectodermally derived cells that give rise to the peripheral nervous system and pigment. This exceptionally high stem cell potential has puzzled researchers for decades and opposing hypotheses to explain how the stemness is gained have been recently presented. Here, using the chicken embryo as our model, we performed a thorough stage by stage monitoring of changes in stemness during the ectodermal patterning process when the three distinct spatially restricted domains, which commit to the future skin, neural crest, and the central nervous system form during the time from gastrulation to end of neurulation. For our analysis, we used three independent high-resolution approaches including our custom designed imaging and machine-learning based quantitative single-cell and single-molecule level highly Multiplex Spatial Transcriptomics technique (analysis of simultaneous co-expression of 35 genes in individually segmented cells in intact in vivo tissues) complemented with bulk and single cell RNA-sequencing. Unexpectedly, we find undecided pan-ectodermal stem cells with a pluripotent gene expression profile spanning the entire ectoderm much later in the neurulation process than previously anticipated. In line with this, we find that ectoderm patterning is not completed before the end of neurulation when high stemness, as evaluated both spatially and transcriptionally, only remains in the neural crest domain. Our results propose a novel mechanism for how stemness is gained and maintained in

the neural crest, and furthermore, challenge how we understand gastrulation.

Funding Source: NIH DE000748-04, Academy of Finland, Sigrid Juselius Foundation, Väre Foundation
Keywords: neural crest stemness formation, gastrulation, ectoderm patterning

TOPIC: EPITHELIAL_GUT

608

THE ROLE OF ARID3A IN MAINTENANCE OF INTESTINAL EPITHELIAL HOMEOSTASIS

Angelis, Nikolaos - *Stem Cell and Cancer Laboratory, Francis Crick Institute, London*
Baulies, Anna - *Stem Cell and Cancer Biology Laboratory, Francis Crick Institute, London, UK*
Kucharska, Anna - *Stem Cell and Cancer Biology Laboratory, Francis Crick Institute, London, UK*
Kelly, Gavin - *Bioinformatics and Biostatistics Science Technology Platform, Francis Crick Institute, London, UK*
Llorian Sopena, Miriam - *Bioinformatics and Biostatistics Science Technology Platform, Francis Crick Institute, London, UK*
Li, Vivian - *Stem Cell and Cancer Biology Laboratory, Francis Crick institute, London, UK*

Abstract: Intestinal stem cells (ISCs) reside at the bottom of intestinal crypts and give rise to progenitor cells (+4/+5 cells), which eventually differentiate into various mature epithelial cell types. Wnt, Notch and Tgf-β/Bmp signalling pathways form gradient of expression along the crypt-villus axis and play a central role in regulating ISC homeostasis and lineage commitment. Despite the good understanding of the signalling pathways involved in ISC self-renewal and fate decision, the underlying mechanism of the dynamic lineage selection and plasticity of the +4/+5 early progenitors remains largely unknown. Here, we identify Arid3a as a novel regulator of epithelial cell differentiation and maturation. Arid3a is up-regulated once ISCs exit their niche and start migrating above the +4 position and it shows a remarkable expression gradient that accumulates at the tip of the villus. Using a combination of in vivo and in vitro tools, we show that Wnt signalling has an inhibitory role on expression of Arid3a, while Tgf-β signalling promotes its expression. Intestinal epithelial-specific deletion of Arid3a drives up-regulation of enterocyte programme of the upper villus and results in increased absorption and energy metabolism. Interestingly, the enterocyte antimicrobial programme at the inter-villus junction remains largely unaffected. Arid3a-deficient mice also exhibit a reduced number of proliferative transit-amplifying cells at the upper crypt, where number of stem cells is not changed. We conclude that Arid3a drives maintenance of epithelial homeostasis across the crypt villus axis and tightly regulates terminal maturation of epithelial enterocytes as they migrate towards the tip of the villus.

Keywords: Intestinal stem cells, Homeostasis, Differentiation

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PAAC IT UP: INTRAVITAL LIVE IMAGING OF FUNCTIONING ADULT INTESTINES REVEALS DYNAMIC EXTREMES OF BRUSH BORDER REGENERATION

Galenza, Anthony - *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA, USA*
Su, Elsa - *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA, USA*
Moreno-Roman, Paola - *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA, USA*
Acosta-Alvarez, Lehi - *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA, USA*
Kolotueva, Irina - *Faculté de biologie et médecine, Universite de Lausanne, Switzerland*
O'Brien, Lucy - *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA, USA*

Abstract: Rapid replacement of damaged cells is essential for successful regeneration of injured tissues. Much is known about the injury signals that activate regenerative stem cell divisions, but how injury-born cells accelerate terminal differentiation to restore homeostasis is unknown. For barrier epithelia such as the mammalian airway and olfactory lining and the Drosophila intestine, this challenge is acute because stem cells and newborn progeny lack a protective apical membrane. Using Focused Ion Beam-Scanning Electron Microscopy of the adult Drosophila intestine, we find that stem cell progeny generate their future apical membrane, or brush border, via a deep plasma membrane invagination we call a Pre-Assembled Apical Compartment (PAAC). Using intravital imaging, we examine how transient injury impacts PAAC formation and brush border regeneration. Upon acute ingestion of the toxin bleomycin, or genetic ablation of brush border homeostasis, the ordered architecture of the brush border is rapidly disrupted. In response, stem cell progeny form a profusion of miniature PAACs (multi-PAACs) that both cluster and extend linearly across their surface, suggesting a frantic attempt to seal gaps from lost cells. Within 48 hours of toxin removal, brush border restoration is complete. Activation of the injury-associated JAK-STAT pathway in stem cell progeny triggers multi-PAAC formation in otherwise healthy guts, which shows that the regenerative response does not require tissue damage. We suggest that multi-PAACs are an emergency stopgap deployed by injury-activated stem cell progeny to withstand premature exposure to the intestine's lumenal contents.

Funding Source: This work was supported by NIH R01GM116000-01A1, NIH R35GM141885-01, and ACS RSG-17-167-01 to L.E.O.
Keywords: Tissue regeneration, Brush border, Intravital imaging

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PCLAF/PAF-ACTIVATED THE DREAM COMPLEX TRANSCRIPTOME IS INDISPENSABLE FOR ALVEOLAR CELL LINEAGE PLASTICITY FOR LUNG REGENERATION

Kim, Bongjun - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Huang, Yuanjian - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Ko, Kyung-Pil - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Jun, Sohee - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Zhang, Shengzhe - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Zou, Gengyi - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Zhang, Jie - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*
Little, Danielle - *Developmental Neurobiology, St. Jude Children's Research Hospital, Houston, TX, USA*
Vila, Lisandra - *Pulmonary Medicine, MD Anderson Cancer Center, Houston, TX, USA*
Paschini, Margherita - *Stem Cell Program, Boston Children's Hospital Harvard Medical School, Boston, MA, USA*
Chen, Jichao - *Pulmonary Medicine, MD Anderson Cancer Center, Houston, TX, USA*
Kim, Carla - *Stem Cell Program, Boston Children's Hospital Harvard Medical School, Boston, MA, USA*
Park, Jae-Il - *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX, USA*

Abstract: The spatiotemporal orchestration of lung stem/progenitor cells is essential for lung regeneration, of which failure leads to lung disease, including fibrosis. We sought to identify mechanisms controlling lung stem/progenitor cells during lung regeneration. We previously found that PCLAF/PAF/KIAA0101 remodels the DREAM complex for cell quiescence exit and cell proliferation. PCLAF is expressed explicitly in the pulmonary proliferative cells (PPC), along with the DREAM target genes. Pclaf expression and Pclaf-expressing cells were acutely increased upon lung injury. Intriguingly, Pclaf knock-out mice exhibited lung fibrosis with impaired alveolar regeneration, resulting from the loss of alveolar type I cells. The single-cell transcriptome and organoid analyses showed that Pclaf transactivated the DREAM target gene expression for the repopulation of alveolar type I cells from alveolar type 2 cells. Additionally, pharmacological mimicking of the Pclaf-mediated transcriptome markedly increased alveolar regeneration in vitro and in vivo, without neoplasia. Together, our study unveiled the pivotal roles of the PCLAF-DREAM axis in controlling stem/progenitor cell activation and cell lineage, further proposing the potentially viable option for lung regenerative medicine.

Funding Source: CPRIT (RP200315), NCI (CA193297, CA256207)
Keywords: LUNG REGENERATION, PCLAF/PAF/KIAA0101, DREAM COMPLEX

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EXPLORING THE CELLULAR AND MOLECULAR MECHANISMS CONTROLLING STRETCH-MEDIATED TISSUE EXPANSION

Aragona, Mariaceleste - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Aguilera Stewart, Caroline - *Novo Nordisk Foundation Center for Stem Cell Biology, reNEW, University of Copenhagen, Denmark*
Mai Nguyen, Tram - *Novo Nordisk Foundation Center for Stem Cell Biology, reNEW, University of Copenhagen, Denmark*

Abstract: Stretch-mediated tissue expansion is commonly used to grow extra skin for reconstructive surgery. To ensure harmonious growth, the two main skin compartments, the epidermis and the dermis, must both adjust their behaviour. It remains unexplored which mechanisms allow stretch-mediated tissue expansion on the dermis and in particular in fibroblasts, the main cell type in the dermis. To study the temporal consequences of stretching the skin in vivo, we recently developed a mouse model that uses miniaturised prosthesis placed subcutaneously on mouse back skin. With this model and our expertise in mechanobiology and in analysing cell fate dynamics, we will be able to understand how fibroblasts cope with stretching. Here we use lineage tracing, proliferation kinetics and whole mount imaging analysis to define the cellular mechanisms involved in tissue expansion. Single-cell gene expression and epigenetic profiling will elucidate the gene regulatory networks that orchestrate tissue expansion.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729).
Keywords: skin expansion, mechanobiology, cell fate

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ONCOGENIC KRAS INDUCES SPATIOTEMPORALLY SPECIFIC TISSUE DEFORMATION THROUGH CONVERTING FLUCTUATED INTO SUSTAINED ERK ACTIVATION

Xin, Tianchi - *Genetics, Yale School of Medicine, New Haven, CT, USA*
Regot, Sergi - *Molecular Biology and Genetics, Johns Hopkins School of Medicine, Baltimore, MD, USA*
Greco, Valentina - *Genetics, Yale School of Medicine, New Haven, CT, USA*

Abstract: Tissue regeneration and maintenance rely on coordinated stem cell behaviors. This orchestration can be impaired by oncogenic mutations leading to tissue architecture disruption and ultimately cancer formation. However, it is still largely unclear how oncogenes perturb stem cells' functions to break tissue architecture. Here, we utilized intravital imaging and novel signaling reporter to investigate the cellular and molecular mechanisms by which oncogenic Kras mutation causes tissue disruption in the hair follicle. Through longitudinally tracking the same hair follicles in live mice, we found KrasG12D, a mutation that can lead to squamous cell carcinoma, induces epithelial tissue deformation in a spatiotemporally specific manner. This tissue architecture abnormality is linked with a spatial dysregulation of stem cell proliferation and migration during hair follicle regeneration. By

using a reporter mouse that allows us to capture real-time ERK signal dynamics at the single cell level in live animal, we discovered that KrasG12D converts the transient ERK signal fluctuation in the stem cells into sustained activation. In contrast, hair follicles carrying oncogenic mutation HrasG12V, which does not cause tissue deformation, still exhibit fluctuated ERK activation. Furthermore, by combining drug treatment with longitudinal hair follicle imaging, we demonstrated that inhibiting ERK signal reverts the KrasG12D-induced tissue deformation, suggesting the alteration of the ERK signal dynamics led to tissue architecture disruption in Kras mutant hair follicles. Intriguingly, we also found that low number of KrasG12D cells are insufficient to induce sustained ERK activation and deform tissue, suggesting a collective effect from a large group of mutant cells is required to disrupt the tissue. Altogether, our work supports a niche-dependent mechanism for oncogene-induced tissue architecture disruption. Oncogenic mutations induce tissue abnormalities when spatiotemporally specific conditions are met, which allows mutant stem cells disturb local cell coordination through altering dynamic signal communications.

Keywords: Hair follicle regeneration, Oncogenic Ras mutation, ERK signaling dynamics

TOPIC: GERMLINE

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SPERMATOGONIAL STEM CELLS CRYOPRESERVED FOR OVER TWENTY YEARS SUCCESSFULLY REGENERATE SPERMATOGENESIS

Whelan, Eoin C. - *Biomedical Science, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Fan, Yang - *Histology and Embryology, Yangzhou University, Yangzhou, China*
Avarbock, Mary - *Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Sullivan, Megan - *Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Beiting, Daniel - *Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Brinster, Ralph - *Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*

Abstract: Treatment of cancer in children is increasingly successful but leaves many prepubertal boys suffering from infertility or subfertility later in life. A current strategy to preserve fertility in these boys is to cryopreserve a testicular biopsy prior to treatment with the expectation of future technologies allowing for the reintroduction of stem cells and restoration of spermatogenesis. The objectives of this study were to determine if rat spermatogonial stem cells cryopreserved for 23+ years could successfully re-establish spermatogenesis in an infertile host, and if so, whether spermatogenesis was affected by the process. We demonstrated that rat spermatogonial stem cells frozen for over 23 years can be transplanted into recipient mice and produce all differentiating germ cell types. However, compared with freshly-isolated cells or those frozen for a short period of time (< 4 months), long-frozen cells do not colonize efficiently and histological analysis showed reduced production of spermatids. Single cell RNA sequencing revealed similar profiles of gene expression changes in spermatogonial stem cells between short and long-frozen cells as compared with fresh immediately after thawing (n=3). Conversely, following transplantation, long-frozen samples showed enhanced stem cell signaling in the undifferentiated spermatogonia compartment, consistent with self-renewal and a lack of differentiation. For each treatment, both unselected and EpCAM+ samples enriched for spermatogonia were analyzed (n=3). In addition, long-frozen samples showed fewer round spermatids with detectable protamine expression, suggesting a partial block of spermatogenesis after meiosis resulting in a lack of elongating spermatids. These findings strongly suggest that prolonged cryopreservation can impact the success of transplantation to produce spermatogenesis, which may not be revealed by analysis of the cells immediately after thawing. Our analysis uncovered persistent effects of long-term freezing via functional regeneration of the tissue and this phenomenon must be accounted for any future therapeutic application.

Funding Source: Robert J. Kleberg, Jr and Helen C. Kleberg Foundation
Keywords: Spermatogonial stem cells, Cryopreservation, Single-cell RNA sequencing

TOPIC: HEMATOPOIETIC SYSTEM

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MECHANISMS OF DYNAMIC HEMATOPOIETIC STEM CELL RECONSTITUTION CAPACITY THROUGHOUT LIFE

Worthington, Atesh - *Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA, USA*
Poscablo, Donna - *Molecular, Cell and Developmental Biology, University of California Santa Cruz, CA, USA*
Cool, Taylor - *Molecular, Cell and Developmental Biology, University of California Santa Cruz, CA, USA*
Rodriguez y Baena, Alessandra - *Molecular, Cell and Developmental Biology, University of California Santa Cruz, CA, USA*
Martin, Eric - *Molecular, Cell and Developmental Biology, University of California Santa Cruz, CA, USA*
Forsberg, Camilla - *Biomolecular Engineering, University of California Santa Cruz, CA, USA*

Abstract: Hematopoietic stem cells (HSCs) reconstitution capacity is dynamic throughout life, specifically with regards to tissue-resident immune cells. Unlike circulating immune cells that are continuously generated from HSCs, many tissue resident immune cells are of fetal origin and poorly generated from adult HSCs. Therefore, to improve the clinical utility of HSCs, it is essential to understand how tissue-resident immune cells are generated. We previously demonstrated that tissue-resident macrophages and lung eosinophils surprisingly require the lymphoid associated gene, IL7Rα, for their generation. This led us to interrogate IL7Rα's as well as Flk2's role in tissue-resident lymphoid cells (TLCs). Using Il7r- and Flk2-Cre lineage tracing, we found that peritoneal B1a cells, splenic marginal zone B (MZB) cells, lung ILC2s and regulatory T cells (Tregs) were highly labeled. Despite high labeling, loss of Flk2 minimally affected the generation of these cells. In contrast, loss of IL7Rα, dramatically reduced the number of B1a cells, MZBs, ILC2s and Tregs, both in situ and upon transplantation, indicating an intrinsic and essential role for IL7Rα. Surprisingly, reciprocal transplants of wild-type HSCs showed that an IL7Rα−/− environment selectively impaired reconstitution of TLCs when compared with TLC numbers in situ. To further understand differential TLC reconstitution capacity of fetal and adult HSCs,

we competitively transplanted fetal and adult HSCs. Despite similar numbers of engrafted HSCs, fetal HSCs outcompeted adult HSCs in B1a and MZB cell reconstitution. Taken together, our data defined Flk2- and IL7Rα-positive TLC differentiation paths and revealed functional roles of Flk2 and IL7Rα in TLC establishment. Importantly, although adult HSCs having reduced TLC potential, their ability to reconstitute circulating lymphoid cells is unchanged. Interestingly, we and others have demonstrated that HSC reconstitution capacity is reduced upon aging, despite a dramatic expansion HSCs. These functionally poor HSCs may serve as potential therapeutic source if they can be manipulated to behave like younger HSCs. Therefore, we have begun to test underlying mechanisms of reconstitution potential with the goal of defining regulators of HSC reconstitution capacity to target and improve HSC-based therapies.

Keywords: developmental hematopoiesis, tissue-resident immune cells, transplantation

TOPIC: LIVER

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IGFBP2+ MIDLOBULAR HEPATOCYTES PREFERENTIALLY CONTRIBUTE TO LIVER HOMEOSTASIS AND REGENERATION

Lin, Yu-Hsuan - *Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Abstract: In the liver lobule, heterogeneous hepatocytes can be subdivided into zones based on metabolic gene expression. We previously used a panel of 14 CreER mouse strains to show that midlobular zone 2 hepatocytes are an important cellular source for liver homeostasis and regeneration. However, no existing mouse strain allows for the exclusive fate mapping of zone 2 cells. Here, we generated a new Igfbp2-CreER knockin strain to evaluate the contributions of midlobular hepatocytes which are enriched for IGFBP2 expression. During homeostasis over 1 year, zone 2 hepatocytes labeled by IGFBP2 increased in abundance from occupying 20.8% to 41.2% of the lobule area. The magnitude of change in the IGFBP2 population is less than the HAMP2 population (from 7.4% to 27.4%), suggesting that there is heterogeneity among hepatocytes in zone 2. After either pericentral injury with carbon tetrachloride or periportal injury with DDC, IGFBP2 positive cells replaced lost hepatocytes in zone 3 and zone 1, respectively. IGFBP2 positive cells also preferentially contributed to regeneration after 70% partial hepatectomy. In addition, we detected a significant increase of labeled midlobular hepatocytes during pregnancy induced liver growth. Taken together, these studies demonstrated the contribution of IGFBP2-labeled zone 2 hepatocytes to the maintenance of liver homeostasis and regeneration.

Keywords: Lineage tracing, Liver zonation, Liver regeneration

TOPIC: MUSCULOSKELETAL

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THERAPEUTIC EFFECTS OF SIALYLLACTOSE IN HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS FOR CELL BASED TISSUE ENGINEERING

Baek, Ahreum - *Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Baek, Dawoon - *Department and Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Jo, Seongmoon - *Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*
Kim, Sung Hoon - *Department and Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Cho, Sung-Rae - *Department and Research Institute of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Osteoarthritis (OA) involves cartilage damage, dysfunctional chondrocyte proliferation, and leading cause of disability in humans. Cell-based therapies have been developed to restore cartilage damage and prevent cartilage degeneration. Especially, human bone marrow-derived mesenchymal stem cells (hBMSCs) have self-renewal capacity and the potential for multi-lineage differentiation. However, loss of self-renewal and multi-lineage differentiation potential occurs in long-term in vitro cultivation. Therefore, new techniques require to preserve hBMSCs multipotency after long-term expansion. 3′-Sialyllactose, a natural compound, is present in human milk and exhibits anti-inflammatory properties and modulates immune homeostasis. In this study, we elucidate the potential therapeutic effects of 3'-Sialyllactose on hBMSCs. Continuously treated with 3'-Siallylactose on hBMSCs, the stemness capacity was significantly increased and senescence was significantly decreased compared to those of untreated hBMSCs. Next, to induce chondrogenesis on hBMSCs, 3-Siallylactose was treated continuously with chondrogenesis differentiation medium, and micromass were conducted. The expression levels of chondrogenic markers were significantly increased and the expression levels of hypertrophic markers were significantly decreased in 3'-Siallylactose treated hBMSCs compared to untreated hBMSCs. Taken together, these effects suggest that 3'-Siallylactose is considered as a natural therapeutic agent for cell-based tissue engineering.

Funding Source: This research was funded by the NRF (2021R1F1A1062829 and 2021R1A6A3A01086535), KHIDI (HI21C1314), and KFRM (21A0202L1 and 21C0715L1).
Keywords: Osteoarthritis, Human bone marrow-derived mesenchymal stem cells, Sialyllactose

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CATHEPSIN K MARKS DISTINCT, LONG-TERM PERIVASCULAR CELLS WITH CHARACTERISTICS OF SKELETAL STEM AND PROGENITOR CELLS IN THE ADULT MOUSE

Mui, Byron - *Skeletal Biology Section, National Institutes of Health, Bethesda, MD, USA*
Futrega, Kathryn - *Skeletal Biology Section, National Institutes of Health, Bethesda, MD, USA*

Gadomski, Stephen - *Skeletal Biology Section, National Institutes of Health, Bethesda, MD, USA*
Chalut, Kevin - *Wellcome-MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Robey, Pamela - *Skeletal Biology Section, National Institutes of Health, Bethesda, MD, USA*

Abstract: Bone development, homeostasis, and regeneration rely upon the function of skeletal stem and progenitor cells (SSPCs). Seminal studies have identified various subsets of SSPCs, each with varying capacities to differentiate into multiple cell types and self-renew. However, an incomplete understanding of the in vivo identities of SSPCs, their spatio-temporal and lineage relationships, and niche-specific functional properties has slowed progress in harnessing them for therapies. Using a cell lineage tracing mouse model (Cathepsin K (Ctsk)-cre;Rosa26 mT/mG), a previous study showed that in young mice, Ctsk-mGFP labeled SSPCs in the periosteum, the outermost tissue layer of bones. Using the same mouse model, we found that at 4 weeks and beyond, Ctsk-mGFP additionally labeled non-hematopoietic cells in the metaphysis and the central bone marrow. These newly identified Ctsk-mGFP cells demonstrated morphological features of perivascular cells; therefore, we performed 3D deep confocal imaging of whole bones to accurately assess their proximity to vasculature. Indeed, Ctsk-mGFP marked perivascular cells associated with sinusoids and arterioles in the metaphyseal and central marrow, as well as the vasculature in the periosteum. Single-cell RNA sequencing of 8-week-old femoral Ctsk-mGFP cells revealed 4 distinct populations, one of which resembled both SSPCs through expression of stem cell markers (Ly6a, Gsn) and perivascular cells (Cxcl12, Pdgfr-alpha). It also highly expressed several basement membrane genes typical of the perivascular niche. Together, our data show for the first time that Ctsk-mGFP marks perivascular cells in the periosteum and metaphyseal and central marrow with characteristics of SSPCs in their morphology, proximity to blood vessels and gene expression profiles. To functionally test Ctsk-mGFP perivascular cells, we performed stabilized femoral fractures of 8-week-old mice. Initially, Ctsk-mGFP perivascular cells underwent expansion and migration away from blood vessels. After 7 days, they differentiated into chondrocytes before being replaced by regenerated bone, confirming their functional SSPC identity. Further work aims to distinguish the in vivo phenotype and functional differences between Ctsk-mGFP perivascular cells in different skeletal regions.

Funding Source: 1ZIADE000380
Keywords: skeletal stem cell, perivascular, regeneration

TOPIC: NEURAL

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META -ANALYSIS OF SINGLE-CELL RNA-SEQUENCING DATA REVEALS KEY GENES INVOLVED IN MAINTAINING QUIESCENT AND PROLIFERATING NEURAL CELL POPULATIONS IN GLIOBLASTOMA

Vikram, Rajeev - *Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan*
Chou, Wen-Cheng - *Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan*
Shen, Chen-Yang - *Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan*

Abstract: IDH- wildtype glioblastoma (GBM) is a relatively common yet one of the most challenging brain tumors to treat. Tumor growth and treatment resistance in GBM is driven by a small population of cells with stem cell like potential of self-renewal, migration and differentiation. Identifying and characterizing these stem cell-like populations has been particularly challenging owing to marked inter and intra tumoral heterogeneity observed in these tumors. According to the cancer stem cell (CSC) theory, these tumorigenic populations are composed of long-lived cells associated with latency, immune evasion proliferation and metastasis. We performed a meta-analysis of 5 single-cell RNA-sequencing studies of high-grade glioblastomas with 38,117 cells from 53 pediatric and adult tumor samples using comparative integrated analysis of gene expression patterns, cell cycle stages and gene networks. Our results reveal the existence of cycling and quiescent stem like cells in GBMs of all age groups. Quiescent cells were characterized by higher expression of DCX, ELAVL4, SOX4 and SOX11 genes, key regulators of neural progenitor state, whereas cycling cells selectively overexpressed CENPF, CKS2, HMGB2, HMGN2 and TUBA1B, genes involved in proliferation, cell division and the assembly of cell organelles. Quiescent cells from both adult and pediatric groups overexpressed a number of genes for ribosomal proteins indicating an important role of ribosome biogenesis in the maintenance of quiescence state. Comparatively, Pediatric tumors had significantly higher proportion of quiescent cells, whereas some adult tumor samples lacked cells with well defined quiescence characteristics. Through our analysis, we demonstrate the exitance of quiescent and cycling states within stem cell niches in GBM which mimic normal neural progenitor and proliferating states. We identify key genes and biological programs differentiating and modulating the two states. Further research elucidating the role of these genes in GBM will help in identifying stem cell specific therapeutic targets.

Keywords: Glioblastoma, quiescent stem cells, single-cell RNA-sequencing

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HUMAN ENDOTHELIAL CELL CONTACT PROMOTES A PUTATIVE TYPE B CELL PHENOTYPE IN HUMAN NEURAL STEM/ PROGENITOR CELLS

Gutierrez, Brenda - *Anatomy and Neurobiology, University of California, Irvine, CA, USA*
Flanagan, Lisa - *Neurology, University of California, Irvine, CA, USA*

Abstract: Neural stem/progenitor cells (NSPCs) lie in close proximity to blood vessels in the developing brain and in adult brain neural stem cell niches such as the subventricular zone. This adjacency allows for close interaction between NSPCs and the endothelial cells (ECs) that form blood vessels. Deciphering how NSPCs and ECs regulate each other is critical for understanding brain development and regulation of adult stem cell niches. In rodent systems, EC secreted factors regulate NSPC proliferation, differentiation, and self-renewal. However, the effect of EC contact on NSPC phenotype has not been well-studied, especially in humans. We co-cultured human NSPCs (hNSPCs) with human ECs (hECs) and found that hECs impact hNSPC phenotype. Co-culture with hECs stimulates a significant increase in the percentage of cells expressing GFAP, SOX2 and LewisX, which are markers for type B cells, the NSPCs of the adult subventricular zone. The increase in putative type B cells was not seen with hEC conditioned media (CM), indicating hEC contact rather than se-

creted factors promotes a human type B cell phenotype. Single cell RNA sequencing (scRNAseq) of hNSPCs alone, in co-culture with hECs, and in hEC CM was conducted to further characterize these putative type B cells and to identify candidates for hEC contact-mediated mechanisms promoting the observed phenotype. ScRNAseq revealed a cluster of cells expressing type B cell markers including GFAP, SOX2, S100A6 identified in mouse type B cells, and Prominin-1 (CD133). Co-culture with hECs leads to an increase in the percentage of type B cells, a decrease in proliferating cells, and a decrease in astrocyte progenitors compared to hNSPCs alone or cultured with hEC CM. We utilized differential gene expression between hNSPCs alone and those co-cultured with hECs to screen for potential signaling mechanisms. HNSPCs co-cultured with hECs exhibit a higher expression of Notch downstream mediators HEY1 and HES4 compared to hNSPCs alone. Interestingly, hNSPCs co-cultured with hECs also show increased expression of genes associated with vasculature development such as SAT1, PGF, and ID1 compared to monoculture. In summary, hEC contact stimulates an increase in the percentage of human putative type B cells while reducing proliferation and differentiation of hNSPCs.

Funding Source: F31 NINDS (F31NS118845), T32 Training Program in Stem Cell Translational Medicine (T32NS082174), UCI MSTP (T32GM008620), R01 (NS119829), UCI Institute for Clinical and Translational Science (UL1 TR001414)
Keywords: neural, endothelial, contact

TOPIC: PANCREAS

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HOW MUCH SUGAR IS TOO MUCH SUGAR? DELETERIOUS EFFECT OF HIGH GLUCOSE CONCENTRATION ON PDMSCS BIOLOGICAL FUNCTIONING AND ADVERSE EFFECT ON FURTHER PANCREATIC PROGENITOR DIFFERENTIATION

Guerrero, Erika N. - *Regenerative Medicine Deparment, Gorgas Memorial Institute for Health Studies, Panama*
Vega, Shantal - *Regenerative Medicine, Gorgas Memorial Institute for Health Studies, Panama*
Fu, Cindy - *Regenerative Medicine, Gorgas Memorial Institute for Health Studies, Panama*
Solis, Mairim - *Regenerative Medicine, Gorgas Memorial Institute for Health Studies, Panama*

Abstract: Glucose is an essential source of cellular energy, whose chronic elevated concentration results in ß-cell dysfunction and finally induced apoptosis. Furthermore, hyperglycemia leads to oxidative damage in major organs. However, appropriate glucose concentration ensures stable cell maintenance and it's essential for protein and lipid synthesis. Stem cells differentiate into a widespread type of cells in specific inducing media. Production of effective insulin-secreting ß-cell by inducing differentiation has gained attention as an alternative approach for diabetes therapeutics and disease modeling. However, before stem cell therapeutics strategies can translate into human applications, appropriate differentiation protocols including glucose concentration, cell maturation, insulin secretion and functional aspects need to be well-established in vitro. We recently showed placenta-derived mesenchymal stem cells (PDMSCs) from median maternal age donors presented increased proliferation and differentiation capacity, although, effect of glucose concentration may impair cellular functioning. Here, we demonstrate: (a) PDMSCs cultured in increasing concentrations of glucose (2mM, 5mM, 10mM, 20mM) presented increasing proliferative rates, increasing multipotency and increased membrane-associated glucose transporter facilitators (GLUT) expression; up to 30mM glucose concentration where PDMSCs showed significantly reduced proliferation, multipotency and GLUT-expression. (b) PDMSCs differentiation to pancreatic progenitor cells in 20mM glucose promotes rapid differentiation as analyzed by significantly increased Insulin gene expression and pancreatic markers (HNF6, Kkx6.1, PDX1) compared to low glucose. Interestingly, we observed that PDMSCs secretion of IL-6 after differentiation to definitive endoderm and primitive gut is glucose concentration-dependent. Thus this novel study reveals glucose concentration plays a critical role in differentiation of PDMSCs towards ß- cells. Current efforts in our laboratory are focused on exploring avenues for cell transplantation while keeping optimal immunomodulatory protection against host immune response and providing protocols that may prevent adverse effects of glucose on development, maturation and differentiation.

Keywords: Mesenchymal stem cells, Glucose, Immunomodulation

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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HUMAN FETAL MEMBRANE-MESENCHYMAL STROMAL CELLS CAN GENERATE MATURE SPINAL MOTOR NEURONS IN VITRO

Gaggi, Giulia - *Department of Medicine and Aging Sciences, Center of Advanced Studies and Technology, University of "G.D'Annunzio" of Chieti-Pescara, Italy*
Di Credico, Andrea - *Deptartment of Medicine and Aging Sciences, Center of Advanced Studies and Technology, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy*
Di Baldassarre, Angela - *Deptartment of Medicine and Aging Sciences, Center of Advanced Studies and Technology, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy*
Barbara, Ghinassi - *Deptartment of Medicine and Aging Sciences and Center of Advanced Studies and Technology, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy*

Abstract: Human Fetal Membrane-Mesenchymal Stromal cells (hFM-MSCs) are a stem cell population that can be easily isolated from the amniochorionic membrane of term placentas. As all the perinatal stem cells, they might represent a potential good candidate for the regenerative medicine as they are not tumorigenic, have low immunogenicity and their use does not rise any ethical issues. We previously reported that hFM-MSCs share some epigenetic characteristics with induced pluripotent stem cells. Here, we investigated whether the hFM-MSCs can give rise to ectodermal-derived lineages such as spinal motor neurons (MNs). hFM-MSCs were driven toward the MN differentiation by the sequential exposure to specific factors that first inhibit the TGFβ/BMP signaling and then activate the retinoic acid and sonic hedgehog pathways. During the differentiation steps, a gradual gene and protein upregulation of early and late of MN markers (PAX6, HB9, ISL1, the vesicular acetylcholine transporter and neurofilament L) was detected. The electrical maturation of the MNs was monitored by Multi-Electrode Array technology and after 5 weeks the characteristics of the spontaneous electrophysiological activi-

ty (spikes and bursts) were recorded and analyzed. Finally, the microscopical analysis evidenced that, when co-cultured with myotubes, differentiated MNs were able to create neuromuscular junctions and after 7 days of co-colture many areas of robust skeletal muscle cell contractions were observed. In conclusion, our data demonstrated the hFM-MSCs can overcome the mesenchymal restriction, differentiating into cells displaying morphological, phenotypical and functional features of MNs.

Keywords: Human fetal membrane-mesenchymal stromal cells, motor neurons, neuromuscular junction

TOPIC: PLURIPOTENT STEM CELLS

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EFFECTS OF FOCUSED ULTRASOUND ON DRUG TRANSPORTER ACTIVITY IN A FAMILIAL ALZHEIMER'S DISEASE BRAIN ENDOTHELIAL CELL -LIKE MODEL

da Silva Chaves, Juliana C. - *Mental Health and Neuroscience, Queensland Medical Research Institute, Lutwyche, Australia*
Oikari, Lotta - *Cell & Molecular Biology Department, Mental Health Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia*
Wasielewska, Joanna - *Cell & Molecular Biology Department, Mental Health Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia*
Lee, Serine - *Cell & Molecular Biology Department, Mental Health Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia*
White, Anthony - *Cell & Molecular Biology Department, Mental Health Program, QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Abstract: The blood-brain barrier (BBB) is a barrier that plays a role in maintaining homeostasis in the brain. The major function of the BBB is regulating the entry of molecules from the blood to the brain, which is partly controlled by BBB transporters, expressed in brain endothelial cells (BECs). Although the BBB has an important protective function, it simultaneously challenges the delivery of drugs into the brain, in part due to transporter activity. This is a major impediment to drug therapy for brain disorders. In addition, any dysfunction to the BBB can allow the entry into the brain of unwanted substances, leading to increased inflammatory mechanisms and potentially contributing to neurodegeneration, such as Alzheimer's disease (AD). Considering the fact that BBB breakdown may contribute to AD progression, it is crucial to understand the contribution of BBB dysfunction, including BBB transporters to AD pathogenesis. This study aimed to investigate how transporter activity in the BBB is altered in AD patients compared to healthy controls. To achieve this, we used human induced pluripotent stem cells (iPSCs) obtained from AD patients (symptomatic and asymptomatic) carrying the Presenilin 1 mutation (PSEN1), healthy controls and isogenic PSEN1 gene-corrected control lines to derive brain endothelial-like cells (iBECs). Initially, we examined the expression, function, and activity of key BBB transporters in hiPSC- derived iBECs. Subsequently, a treatment to transiently open the BBB was used to enhance drug delivery into the brain. Our results identified differences between familial AD-iBEC and isogenic-controls when compared with unrelated control-iBECs in expression and functional levels of two BBB drug transporters (ABCB1 and ABCC1) commonly associated with AD. Importantly, after the BBB opening treatment using focussed ultrasound, the expression of ABCB1 was significantly increased, with a reduction in expression of ABCC1. This change in expression has been previously associated with increased Aβ accumulation. Our findings demonstrate that PSEN1 mutant AD-iBECs possess the potential to help elucidate the phenotypic differences in the BBB of AD patients compared to healthy ones. Additionally, this in vitro BBB model provides an excellent non-invasive model to further investigate drug delivery into the brain

Keywords: Alzheimer's Disease; Blood-Brain Barrier; Drug Transporters; Functional Assay; Brain Endothelial Cells; hiPSC; BBB disruption; BBB permeability; Transporter Expression; Transporter Activity; Transporter P-glycoprotein; MRP1; BBB Model; Focused Ultrasound; Microbubbles; Amyloid Plaques; Amyloid Clearance, Alzheimer's Disease; Blood-Brain Barrier; Drug Transporters; Functional Assay; Brain Endothelial Cells; hiPSC; BBB disruption; BBB permeability; Transporter Expression; Transporter Activity; Transporter ABCB1 and ABCC1; BBB Model; Focused Ultrasound;, Amyloid Plaques; Amyloid Clearance

TOPIC: EARLY EMBRYO

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CANCER STEM CELLS IN COLORECTAL CANCER

Alhulais, Reem - *School of Pharmacy and Medical Science, Griffith University, GoldCoast, Australia*
Ralph, Stephen - *School of Pharmacy and Medical Science, Griffith University, Gold Coast, Australia*

Abstract: There has been an arousing interest in cancer stem cells (CSCs) ever since it was discovered few decades ago. CSCs are well-known by not only their ability to undergo self-renewal and differentiate into more mature cancer cells but also by their tumour-initiating ability from relatively very small number of cells. Only little investigation into the exact role of isolated populations of (CSCs) has been undertaken and the prevalence of CSCs in malignancies is still a matter of some debate and controversy. Here, we aim to identify specific CSC markers and isolate CSC sub-populations from colon cancer in order to force them from dormancy into active division, which will potentially make them more susceptible to chemotherapy. Expression levels of several colorectal CSCs markers including CD271, SSEA1, EPCAM, Cript-1, or ABCG2 were validated under both hypoxic and normoxic conditions in SW480 and CSC480, colorectal cancer cell lines, using Flow cytometry and immunofluorescence. The relationship between hypoxia and cellular expression of Brn2, which is a transcription factor that could be a CSC marker, was explored via flow cytometry an immunofluorescence. Furthermore, correlation between CSC markers expression in primary and metastasis tissues in human colorectal cancer was examined by immunofluorescence. ABCG2 and Cripto-1 were expressed in low levels on cell-subpopulations compared to CD271, EPCAM or SSEA1. Interestingly, all the markers expression levels were increased in a subpopulation by 72 hours under hypoxia compared to normoxia conditions. However, comparison over the time course of hypoxia; EPCAM, Cripto-1, or ABCG2 expression were decreased at 48 hours and then increased again at 72 hours. The SW480 Brn2-EGFP cell line showed a significant decreased in Brn2 positive cells between the normoxia and hypoxia samples at 24, 48, or 72 hours. We found that all markers were highly expressed in metastasis compared to primary sections in human tissues. ABCG2 and Cripto-1 are potentially suitable markers for studying colon CSCs. Notably, colon CSCs could possibly exert a strong

proportional relationship with hypoxia and metastasis. Additionally, all of the CSC markers were found to be more highly expressed in the metastatic colorectal cancer samples compared with the polyps or primary sections.

Keywords: Cancer stem cells, Colorectal cancer, Markers

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

764

INHIBITORY EFFECTS OF DIABETES ON YOLK-SAC DERIVED ENDO-MACROPHAGE PROGENITORS IN MURINE SKIN

Liyanage, Sanuri R. - *VRC, SAHMRI/Uni Adelaide, Bowden, Australia*
Williamson, Anna - *VRC, SAHMRI/Uni Adelaide, Australia*
Salagaras, Thalia - *VRC, SAHMRI/Uni Adelaide, Australia*
Hassanshahi, Mohammadhossein - *VRC, SAHMRI/Uni Adelaide, Australia*
Primer, Khalia - *VRC, SAHMRI/Uni Adelaide, Australia*
Long, Aaron - *VRC, Department of Cardiology, Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, Australia*
Murphy, Andrew - *Immunometabolism Program, Baker Heart and Diabetes Institute, Melbourne, Australia*
Tan, Joanne - *VRC, SAHMRI/Uni Adelaide, Australia*
Bonder, Claudine - *Centre for Cancer Biology, University of South Australia & SA Pathology, Adelaide, Australia*
Bursill, Christina - *VRC, SAHMRI/Uni Adelaide, Australia*
Psaltis, Peter - *VRC, SAHMRI, Uni Adelaide, Department of Cardiology, Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, Australia*

Abstract: Macrophages and endothelial cells (ECs) play key roles in regulating wound inflammation and neovascularisation. Converging evidence indicates that both cell types share a common embryonic origin from erythromyeloid progenitor cells in yolk sac (YS). We recently discovered YS-derived Lin-CD45+/LoSca-1+c-Kit+CX3CR1+CSF1R+ endothelial-macrophage (EndoMac) progenitors in mouse skin, that are characterised by their ability to form macrophage colony forming units (CFU-M), self-renew and differentiate into ECs and macrophages. Here we studied how high glucose and hyperglycaemia affect their properties as a mechanism for impaired wound healing in diabetes. CFU-M formation from adult C57BL/6 skin digests was decreased 5-fold by exposure to 25 mM glucose in vitro compared to 5 mM glucose. Streptozotocin (STZ)-induced diabetes resulted in 3-fold reduction in skin CFU-M yield and 1.8-fold reduction in the prevalence of skin EndoMac progenitors as measured by flow cytometry. High glucose in vitro and hyperglycaemia in vivo also attenuated the self-renewal of progenitors by 20-fold and 6-fold, respectively and their capacity to produce angiogenic cords in MatrigelTM by 2-fold and 6-fold, respectively. This was associated with reduced ability to form ECs but not macrophages. Csf1rMer-iCre-Mer x RosamT/mG mice that had received tamoxifen in utero to induce GFP expression in YS-derived cells, showed impaired skin wound closure after STZ-induced hyperglycaemia, accompanied by reduced and delayed expansion of YS-derived progenitors compared to non-diabetic wounds. Progenitors from diabetic wounds also displayed reduced proliferative, self-renewal, angiogenic and endothelial forming capacity, along with increased DNA damage and mitochondrial stress. Transfer of EndoMac progenitors from non-diabetic GFP donor skin into wounds of diabetic C57BL/6 mice accelerated wound closure compared to transfer of diabetic progenitors or vehicle control. Non-diabetic progenitors also achieved higher levels of engraftment, self-renewal and differentiation into ECs in vivo than their diabetic counterparts. In conclusion, high glucose inhibits the activity of YS-derived EndoMac progenitors in skin. Therapies targeted at rescuing the reparative properties of these cells may help improve wound healing in diabetes.

Funding Source: NHMRC
Keywords: Yolk-sac (YS) derived progenitors, Diabetes, Endothelial cells and Macrophages

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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SURVEY OF THE ATTITUDE OF THE GENERAL PUBLIC AND PATIENTS IN JAPAN TOWARD RESEARCH INVOLVING THE CULTURE OF HUMAN EMBRYO BEYOND 14 DAYS

Yui, Hideki - *Department of Health Science, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan*
Muto, Kaori - *Department of Public Policy, Institute of Medical Science, University of Tokyo, Minato-ku, Japan*
Yashiro, Yoshimi - *Integrated Research Initiative for Living Well with Dementia, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Itabashi-ku, Japan*
Watanabe, Saori - *Department of Public Policy, Institute of Medical Science, University of Tokyo, Minato-ku, Japan*
Yamagata, Zentaro - *Department of Health Sciences, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan*

Abstract: In the 2021 ISSCR guidelines, research involving the culture of human embryos beyond 14 days (the research) has been removed from the prohibited category. In this study, we examine the characteristics of those who agree or disagree with the research, among the Japanese general public and patients. We conducted an internet-based survey of a general public panel (n=3000) and a patients panel (n=4464). The patients panel included those diagnosed or treated for any disease in the past year. An animated video was used to explain the assumptions of the questions to help respondents understand the questions. While 37.9% of the general public panel and 26.3% of the patients panel agreed with the research, 19.2% of the general public panel and 19.4% of patients panel disagreed, and 42.9% of the general public panel and 54.3% of patients panel could not decide. We performed a logistic regression analysis on the cases that agreed or disagreed, using gender, age, subjective understanding of the video, religion, education, attitudes toward covid19 vaccine, etc., as independent variables. The results showed that in both the panels, males and the vaccine acceptance group agreed more frequently compared to females and the vaccine hesitance group. In the general public panel only, those 50 years and older tended to agree, using the 20s group as a reference category. On the other hand, in the patients panel only, those 60 years and older tended to agree, using 20s group as a reference category. Religion was not a factor in either panel. There are some limitations of the study, such as the skewed age distribution of the patients panel, and that the general panel also included people with bad

subjective health, and the patients panel also included people with good subjective health. In our presentation, we will show the results of some supplemental analysis of opinions about research involving the culture of human embryos within 14 days.

Funding Source: This research was supported by AMED under Grant Number JP21bm0904002
Keywords: culture of human embryo beyond 14 days, attitude of general public and patents in Japan, the 2021 ISSCR guideline

TOPIC: HEMATOPOIETIC SYSTEM

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HEMATOPOIETIC STEM AND PROGENITOR CELL DIFFERENTIATION INTO B CELLS IS REGULATED BY B CELL-PRODUCED GABA VIA A FEEDBACK LOOP

Elujoba-Bridenstine, Adedamola - *Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA*
Tamplin, Owen - *Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI, USA*

Abstract: Hematopoietic stem and progenitor cells (HSPCs) maintain blood production throughout life via self-renewal and multi-lineage differentiation. This makes HSPC transplantation a curative treatment for many hematopoietic diseases. In the bone marrow (BM) niche, many factors and cell types, including the nervous system, regulate HSPC behavior and function. We found that gamma aminobutyric acid (GABA) receptor B subunit 1 (Gabbr1), a neurotransmitter receptor expressed on HSPCs, regulates HSPC proliferation and differentiation into B cells. We also discovered that its ligand, GABA, is also produced in the BM by B cells. We hypothesized that GABA-producing B cells in the BM can regulate their own production via a feedback loop with the Gabbr1 receptor on HSPCs. To test this, we tracked the differentiation of primary mouse HSPCs into B cells over time using the OP9 co-culture system. Using flow cytometry and liquid chromatography-mass spectrometry (LC-MS) of conditioned media, we measured HSPC differentiation and GABA production, respectively, over time. In late-stage co-cultures, there was a dramatic increase in GABA levels in the media, concurrent with an eleven-fold increase in B cells. Addition of Baclofen, a GABBR1 agonist, modulated HSPC differentiation towards a B cell fate. To further investigate the dynamics of B cell differentiation and GABA production, we used genetic tools to delete Gad1 and Gad2, the enzymes responsible for GABA production, specifically in B cells. We crossed Gad1/2-floxed mice to Mb1-Cre mice, deleting these enzymes from the earliest stages of B cell production. Differentiation of Gad-deficient HSPCs in co-cultures produced significantly fewer B cells. We confirmed reduced GABA production during differentiation of Gad-deficient HSPCs using LC-MS of culture media. Addition of GABBR1 agonist partially rescued reduced GABA production and promoted B cell production. Our data suggests that GABA produced by B cells stimulates Gabbr1 receptors on HSPCs to promote further differentiation. This highlights a mechanism for differentiated lineages to regulate their own numbers via communication with upstream stem and progenitor cells.

Funding Source: NIH
Keywords: HSPC, GABA, Regulation

TOPIC: MUSCULOSKELETAL

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HIGH MOBILITY GROUP BOX 1 PROMOTES MSC MIGRATION CAPACITY AND ACCELERATES ANGIOGENESIS DURING DISTRACTION OSTEOGENESIS

Chang, Qi - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Fujio, Masahito - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Tsuboi, Makoto - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Bian, Huiting - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Wakasugi, Masashi - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*
Hibi, Hideharu - *Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Abstract: This study aimed to clarify the effect of high mobility group box 1 (HMGB1) protein on bone healing in a mouse distraction osteogenesis (DO) model. The effects of HMGB1 on the proliferation and migration of human bone marrow mesenchymal stem cells (hMSC) and human umbilical vein endothelial cells (HUVEC) were evaluated in vitro. Tube formation assay was performed for in vitro angiogenesis study. Osteogenic differentiation capacity was evaluated by alkaline phosphatase (ALP) and alizarin red S staining. Furthermore, DO was performed on the mouse tibia. The experiments were divided into three groups: HMGB1 with scaffold implant (HMGB1 group), phosphate-buffered saline with scaffold implant (control group), and no implant group. The new bone and callus formation were evaluated by microcomputer tomography and hematoxylin and eosin staining. Immunofluorescence was used to evaluate angiogenesis on days 10 and 20 and MSC accumulation on day 10. HMGB1 enhanced the migration, mineralization ability, and ALP activity of hMSC and promoted the migration, proliferation ability, and angiogenesis of HUVEC, both in vitro. In addition, the HMGB1 group showed more bone formation than the other two groups and more angiogenesis and MSC. Furthermore, local HMGB1 application promoted bone healing by accelerating angiogenesis and MSC migration during the DO healing process. The results indicate that HMGB1 has a regenerative capacity, as it accelerates angiogenesis and promotes MSC migration that shortens the healing period during DO.

Keywords: Bone regeneration, High Mobility Group Box 1, MSC migration

TOPIC: NEURAL

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EXPLOITING TARGETED EPIGENOME EDITING TO INCREASE YIELD, HOMOGENEITY AND PURITY OF HUMAN IPSC-DERIVED OLIGODENDROCYTE CELLS

Luciani, Marco - *Gene and Neural Stem Cell Therapy for Lysosomal Storage Diseases, San Raffaele Telethon Institute for Gene Therapy, Milan, Italy*
Garsia, Chiara - *GENE AND NEURAL STEM CELL THERAPY FOR LYSOSOMAL STORAGE DISEASES, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy*
Ciccarelli, Eleonora - *GENE AND NEURAL STEM CELL THERAPY FOR LYSOSOMAL STORAGE DISEASES, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy*
Migliara, Alessandro - *EPIGENETIC REGULATION AND TARGETED GENOME EDITING, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy*
Lombardo, Angelo - *EPIGENETIC REGULATION AND TARGETED GENOME EDITING, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy*
Gritti, Angela - *GENE AND NEURAL STEM CELL THERAPY FOR LYSOSOMAL STORAGE DISEASES, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy*

Abstract: The generation of human induced pluripotent stem cells (hiPSCs)-derived oligodendroglial progenitor cells (OPC) and oligodendrocytes (OL) at yield, purity and homogeneity required for advanced biochemical and molecular studies, and for in vivo testing is still a challenge. Several differentiation protocols have been proposed over the years to enhance OPC/OL differentiation from hiPSCs, including those relying on the transient and/or stable overexpression of oligodendroglial Transcription Factors (TFs), which are not without risks of gene misregulation and/or genotoxicity. Our project aims to exploit novel epigenome editing technologies to timely and physiologically modulate the expression of OPC/OL-related genes in hiPSC-derived Neural Stem Cells (hiPS-NSCs). We have selected genes known to drive OPC/OL commitment and we have identified target sites for epigenetic modulators (epieffectors) based on the acetylation and DNA methylation profile of their regulatory regions. The epieffectors are built on catalytically dead Cas9 (dCas9) and fused to enzymatic or scaffolding domains able to activate gene transcription. We demonstrated the capability of targeted epigenome editing to transiently up-regulate OPC/OL-specific transcription factors in hiPS-NSC. By combining transient epigenome editing with established differentiation protocols, we observed an increase in OPC/OL populations yield by FACS and molecular analysis. We are currently assessing if transient epigenome editing is able to increase the myelination capability of hiPS-NSC-derived OL in vitro and in vivo, evaluating the advantages in terms of engraftment, distribution, OL differentiation and myelination capacity in murine models of Leukodystrophies. These experiments will provide proof-of-concept evidence that the transient and physiological modulation of endogenous OPC/OL regulatory network in hiPSC-NSCs allows obtaining high-yield, homogeneous, and pure OL populations to be used for basic and pre-clinical research.

Keywords: Epigenome editing, Oligodendrocyte, human Induced Pluripotent Stem Cells

TOPIC: PLURIPOTENT STEM CELLS

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HUMAN PLURIPOTENT STEM CELL AND CARDIOMYOCYTE DERIVED EXTRACELLULAR VESICLES MEDIATES CARDIAC REGENERATION IN HIGH GLUCOSE INDUCED CARDIAC DAMAGE

Tompkins, Joshua -
Mishra, Pratibha - *AR-DMRI, City of Hope, Duarte, CA, USA*
Lizhar, Elizabeth - *AR-DMRI, City of Hope, Duarte, CA, USA*
Hussey, Deborah - *AR-DMRI, City of Hope, Duarte, CA, USA*
Shokrani, Alireza - *AR-DMRI, City of Hope, Duarte, CA, USA*
Cerneckis, Jonas - *AR-DMRI, City of Hope, Duarte, CA, USA*
Wu, Xiwei - *AR-DMRI, City of Hope, Duarte, CA, USA*
Wang, Jinhui - *AR-DMRI, City of Hope, Duarte, CA, USA*
Riggs, Arthur - *AR-DMRI, City of Hope, Duarte, CA, USA*

Abstract: Individuals with Type 2 Diabetes (T2D) are at a high risk of developing several cardiovascular disorders including coronary and peripheral heart disease, stroke, and a range of cardiomyopathies. Patient derived cardiomyocytes do not proliferate well outside the body and there is a need to better understand the molecular effects hyperglycemia has on human cardiomyocytes. To produce such an in vitro model, we characterized human embryonic/pluripotent stem cell-derived cardiomyocytes (hES/hiPS cell-CMs) exposed to diabetic levels of high glucose. These cardiomyocytes demonstrated: insulin resistance, metabolic shifts, lipotoxicity, hypertrophy, changes in transcriptional signaling, and altered cardiomyocyte (CM) functionality. As Extracellular Vehicles (EVs) have recently been recognized to be of high importance for cell-to-cell communication, and play important roles in controlling cell division, differentiation, and apoptosis. Therefore, we tested pluripotent and CM-derived EVs for protecting and restoring CMs damaged by diabetic conditions. As the human heart has little regenerative capacity, EV-mediated paracrine communication might offer meaningful insights into a working repair strategy for CMs, the primary contractile cell of the heart. Our data clearly indicates both pluripotent and CM-derived EVs to possess anti-hypertrophic and anti-apoptotic properties on CMs exposed to persistent high glucose with ES-EVs demonstrating greater effects. ES EV treatment tend to increase mitochondrial respiration, and further increase the HG associated mitochondrial numbers. Overall, these findings provide insights into the cardioprotective mechanisms of ES and CM derived EVs and suggest that the ES EVs might be used as a tool to understand and promote mechanisms of cardiac healing or regeneration.

Keywords: Human Embryonic Stem Cells, hES/iPS differentiated Cardiomyocytes High Glucose cardiac damage Cardiac regeneration, Extracellular vesicles

TOPIC: HEMATOPOIETIC SYSTEM

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LONG-TERM MULTILINEAGE BLOOD RECONSTITUTION OF HEMATOPOIETIC STEM CELLS WITH HIGH MITOCHONDRIAL POTENTIAL

Fawaz, Malak - *Hematology/Oncology, University Hospital Frankfurt, Frankfurt Am Main, Germany*
Yu, Weijia - *Hematology/Oncology, University Hospital Frankfurt, Frankfurt am Main, Germany*

Sijmonsma, Tjeerd - *Hematology/Oncology, University Hospital Frankfurt, Frankfurt am Main, Germany*
Guenther, Stefan - *CPI - DNA & RNA Technologies, Max-Planck-Institute for Heart- and Lung Research, Bad Nauheim, Germany*
Milsom, Michael - *Division of Experimental Hematology, German Cancer Research Center (DKFZ), Heidelberg, Germany*
Schroeder, Timm - *Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland*
Bibli, Sofia - *Institute for Vascular Signaling, Centre for Molecular Medicine, Goethe University, Frankfurt, Germany*
Rieger, Michael - *Hematology/Oncology, University Hospital Frankfurt, Frankfurt am Main, Germany*

Abstract: Long-term repopulating hematopoietic stem cells (LT-HSCs) are of clinical importance for cellular therapy and regenerative medicine due to their unique characteristics of self-renewal and multipotent differentiation to reconstitute the whole blood system. Recently, mitochondrial metabolism has emerged as an important, yet debatable key player in the regulation of LT-HSCs' stemness, proliferation, and fate decision. However, less attention is paid to multipotent progenitors (MPPs) despite their critical role in sustaining hematopoietic homeostasis. Using the non-invasive dye JC-1, we separate LT-HSCs and MPPs based on their mitochondrial potenial in two metabolically distinct populations: mito-high and mito-low. Whereas only a small subset of LT-HSCs is mito-high (13%), these cells exhibit an extensive expansion capacity over mito-low counterparts in vitro. However, their differentiation pattern remains unaffected. Importantly, serial transplantation experiments demonstrate a multilineage long-term reconstitution potential of mito-high LT-HSCs over 63 weeks with a superiority in leukocyte, erythrocyte and platelet production. Furthermore, RNA-seq and mass spectrometry display unique profiles for mito-high and –low LT-HSCs and MPPs. Mito-high LT-HSCs are transcriptionally more active than mito-low counterparts, and genes involved in cell cycle but not in stemness are upregulated. Additionally, mito-high LT-HSCs present a high glycolytic and respiratory energy metabolism which exceeds mito-low LT-HSCs and both MPP subpopulations. Similar to LT-HSCs, the majority of MPPs is mito-low (73%). Nonetheless, unlike LT-HSCs, mito-low MPPs are more potent in blood reconstitution than mito-high MPPs and can even repopulate tertiary recipients, supplying a reservoir of MPPs with extended long-term reconstitution potential. Our results identify subpopulations of LT-HSCs and MPPs with distinct metabolic states and demonstrate that mitochondrial metabolism is not associated with compromised long-term fitness of LT-HSCs.

Keywords: Hematopoietic stem cells (LT-HSCs), Mitochondrial metabolism, Self-renewal and fate decision

THURSDAY, 16 JUNE, 2022

POSTER SESSION II: ODD

3:00 PM – 4:00 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

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A NOVEL TRANSCRIPTION FACTOR COMBINATION FOR DIRECT REPROGRAMMING TO A SPONTANEOUSLY CONTRACTING HUMAN CARDIOMYOCYTE-LIKE STATE

Romero Tejeda, Marisol - *Pharmacology, Northwestern University, Redmond, USA*
Fonoudi, Hananeh - *Pharmacology, Northwestern University, Chicago, IL, USA*
DeKeyser, Jean-Marc - *Pharmacology, Northwestern University, Chicago, IL, USA*
Weddle, Carly - *Pharmacology, Northwestern University, Chicago, IL, USA*
Magdy, Tarek - *Pharmacology, Northwestern University, Chicago, IL, USA*
Epting, Conrad - *Departments of Pediatrics and Pathology, Lurie Children's Hospital, Chicago, IL, USA*
Burridge, Paul - *Pharmacology, Northwestern University, Chicago, IL, USA*

Abstract: The reprogramming of somatic cells to a spontaneously contracting cardiomyocyte-like state using defined transcription factors has proven successful in mouse fibroblasts, but has been less successful with human cells, limiting the potential clinical applicability of this technology in regenerative medicine. We hypothesized that this issue is due to a lack of cross-species concordance between the required transcription factor combinations. Using the Mogrify algorithm, we identified new candidate transcription factor combinations to induce cell conversion between human fibroblasts and cardiomyocytes. We developed an automated, high-throughput method for screening transcription factors, small molecules, and growth factors utilizing acoustic liquid handling and high-content kinetic imaging cytometry. Using this high-throughput platform, we screened 4,960 unique transcription factor combinations using 24 primary human cardiac fibroblast samples and identified the combination of MYOCD, SMAD6, and TBX20 (MST) that can produce 40% TNNT2+ cells and consistently produce spontaneous cardiomyocyte-like calcium transients and spontaneous contraction at just 25 days. These findings indicate that human cardiac direct reprogramming is feasible at similar levels to that achieved in the mouse and represents a step forward towards the possible clinical application of this direct cardiac reprogramming approach.

Keywords: direct reprogramming, cell fate conversion, regeneration

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CROSS-LINEAGE POTENTIAL OF ASCL1 UNCOVERED BY COMPARING DIVERSE REPROGRAMMING REGULATOMES

Wang, Haofei - *MHI, University of North Carolina at Chapel Hill, NC, USA*
Keepers, Benjamin - *MHI, University of North Carolina at Chapel Hill, NC, USA*
Liu, Jiandong - *MHI, University of North Carolina at Chapel Hill, NC, USA*
Qian, Li - *MHI, University of North Carolina at Chapel Hill, NC, USA*

Abstract: Direct reprogramming has revolutionized the fields of stem cell biology and regenerative medicine, making it possible to interconvert somatic cell fates without intermediary pluripotency. Although studies have identified different transcription factor (TF) cocktails that can reprogram fibroblasts into different cell types, the common mechanisms governing how reprogramming cells undergo transcriptome and epigenome remodeling (i.e., regulatome remodeling) have not been investigated. Here, by characterizing early changes in the regulatome of three different types of direct reprogramming – induced neurons, induced hepatocytes, and induced cardiomyocytes – we identify lineage-specific features as well as common regulatory transcription factors that act in a context-dependent manner for each type of reprogramming. Of particular interest, we discover that the neuronal factor Ascl1 possesses cross-lineage potential; together with Mef2c, it navigates reprogramming fibroblasts through a targeted and potent iCM trajectory. Single-cell multi-omics reveal that A+M reprogramming terminates in a more mature iCM phenotype than MGT. Finally, through ChIP-seq and RNA-seq, we find that Mef2c drives the shift in Ascl1 binding away from neuronal genes towards cardiac genes, guiding their co-operative epigenetic and transcription activities. Together, these findings demonstrate the existence of common regulators of different direct reprogramming processes and argue against the premise that TFs possess only lineage-specific capabilities for altering cell fate – the basic premise used to develop direct reprogramming approaches.

Funding Source: This work was supported by AHA Postdoctoral Fellowship 927906 to H.F.W; NIH T32HL069768 and F30HL154659 to B.K.; AHA20EIA35310348 NIH/NHLBI R35HL155656 to L.Q, NIH/NHLBI R01HL139976 and R01HL139880; AHA 20EIA35320128 to J.L.
Keywords: Cardiac reprogramming, Epigenetics, Direct reprogramming

TOPIC: EARLY EMBRYO

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TREHALOSE PREVENTS PORCINE PREIMPLANTATION EMBRYOS FROM DOUBLE-STRAND BREAKS TO PROMOTE PROPER MITOTIC DIVISION AND CLEARANCE OF MATERNAL MATERIALS

Cai, Lian - *College of Veterinary Medicine, Chungbuk National University, Cheongji, Korea*
Hyun, Sang-Hwan - *College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Autophagy is a self-eating intercellular mechanism that eliminates misfolded proteins and maintains proteostasis. It's been possibly linked to the maternal to zygotic transition (MZT) process of embryonic development. Trehalose, a non-reducing disaccharide, has been proven in multiple neurodegenerative disease models to be a new mechanistic target of rapamycin (mTOR)-independent autophagy enhancer. The purpose of this study was to look into the effects of trehalose supplementation on the developing capability of swine parthenogenetic activated (PA) embryos during in vitro culture (IVC). To accomplish so, the cleavage rate, blastocyst formation rate, total cell quantity, and apoptotic nuclei of blastocysts were measured. Furthermore, the integrity of DNA double-strand was evaluated by γH2A.X staining on one-cell stage embryos and the transcription levels of mitotic checkpoint complex (MCC)-related and MZT-related genes were determined on day 2 and day 7 embryos, respectively, by qRT-PCR analysis. First, we found that trehalose dramatically improved the quality of blastocysts and increased the growth potential of PA embryos by lowering the number of apoptotic cells. Second, we discovered that the DNA double-strand break was suppressed and the period of mitotic division in embryos was delayed, as well as the transcription levels of MCC-related genes were reduced with trehalose supplementation. At last, maternal genes (Zar1, BMP15, and GDF9) were downregulated by trehalose. Taken together, this study suggests that trehalose facilitated the proper mitotic chromosome segregation via preventing DNA double-strand from breakage; thereby contributing to MZT, and finally improving the quality of PA blastocysts.

Funding Source: This work was supported by grants from the "NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)" and "IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.
Keywords: Trehalose, embryo, DNA double-strand break, development potential

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REDOX REGULATION AND THIOL SWITCHES IN CELL DIFFERENTIATION AND VERTEBRATE EMBRYONIC DEVELOPMENT

Breus, Oksana - *Department of Dermatology, University of Colorado, Aurora, CO, USA*
Ruppert, Thomas - *CFMP, Zentrum für Molekulare Biologie der Universität Heidelberg, Heidelberg, Germany*
Kogut, Igor - *AMC, The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Denver, Aurora, CO, USA*
Dickmeis, Thomas - *IBCS-BIP, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany*

Abstract: Cell differentiation in embryogenesis is a complex process orchestrated by multiple regulatory mechanisms. Recent studies indicate that metabolism of pluripotent and differentiated cells significantly differs. Pluripotent cells possess poorly developed mitochondria and rely on glycolysis for their energetic needs. In differentiated cells, mitochondria are highly active. Such metabolic shift is essential for cell differentiation. Mitochondria is a major source of intracellular reactive oxygen species (ROS), which impact embryogenesis presumably through reversible oxidation of susceptible thiol groups on cellular proteins. However, the regulation of embryogenesis by ROS remains poorly understood. We visualized and quantified intracellular dynamics of hydrogen peroxide (H2O2), with new ultrasensitive genetically encoded redox reporters (roGFP2-tsa2 and Hyper7) targeted to cytoplasm or mi-

tochondria of live zebrafish embryos. We also estimated changes in redox couples that modulate intracellular ROS content (GSH/GSSG, NADPH/NADP, NADH/NAD) in development. The analysis revealed a steep increase in cytoplasmic H2O2 starting at mid somitogenesis (16-18hpf), which coincides with a start of terminal differentiation in somites and other embryonic tissues. Life imaging of mitochondria demonstrated dramatic changes in mitochondria morphology and dynamics at this time point, indicating a possible causal link between these processes. We therefore asked whether observed ROS elevation causes a molecular print on cellular proteome by modifying cysteine residues via their thiol groups. We have developed a pipeline for analysis of thiol proteome on different stages of zebrafish embryogenesis with iodoTMT isobaric labels. Obtained MS/MS spectra revealed that mid somitogenesis is characterized by a global shift toward the more oxidized state of redox sensitive cysteines on hundreds of proteins that functionally cluster around processes critical for cell differentiation and tissue patterning such as protein biosynthesis and degradation, mRNA splicing and maturation, DNA replication and chromatin modifications, as well as metabolism. The functional relevance of these modifications and their possible roles as thiol switches during vertebrate development remains to be established.

Funding Source: DFG Priority Program1710 “Dynamics of Thiol-Based Redox Switches in Physiology”.
Keywords: Redox signaling, Embryonic development, Metabolism

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NEUROTROPHIN-4 ACTIVATES ERK1/2 SIGNALING PATHWAY IN CUMULUS CELLS AND IMPROVES OOCYTE QUALITY DURING IN VITRO MATURATION OF PORCINE OOCYTES

Kim, Mirae - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*
Hyun, Sang-Hwan - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Neurotrophin-4 (NT-4), a granulosa cell-derived factor and a member of the neurotrophin family, is known to promote follicular development and oocyte maturation in mammals. The aim of this study was to investigate the effects and its specific mechanism of NT-4 on in vitro maturation (IVM) of porcine oocytes. We confirmed specific gene transcription levels in NT-4-treated oocytes after IVM. The mRNA expression levels of oocyte-secreted factor genes (GDF9 and BMP15), sperm-oocyte interaction-related gene (CD9), and DNA methylation-related gene (DNMT3A) were significantly higher in NT-4 (10 ng/mL)-treated oocytes than in the control. We also identified the protein expression levels of total extracellular signal regulated protein kinase (ERK1/2) and phosphorylated (P)-ERK1/2 to further investigate the mechanisms involved in the downstream signaling pathway of NT-4/TrkB in NT-4-treated cumulus-oocyte complexes during IVM. The P-ERK1/2 protein level was significantly increased in NT-4-treated cumulus cells during IVM, whereas there was no significant difference in NT-4-treated oocytes during IVM. Moreover, we examined the developmental potential of NT-4-treated oocytes during IVM after somatic cell nuclear transfer (SCNT). There was no significant difference of cleavage rates, but the NT-4-treated group showed a significantly higher blastocyst formation rate and total cell numbers than control (21.5 ± 2.8 % and 61.7 ± 1.0 vs 33.8 ± 1.6 % and 78.7 ± 5.5, respectively). Taken together, these results demonstrated NT-4 activates ERK1/2 signaling pathway in cumulus cells and improves oocyte quality during IVM of porcine oocytes. Furthermore, supplementation with NT-4 during IVM promotes subsequent developmental potential of SCNT embryos.

Funding Source: This work was supported by grants from the “NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)” and “IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)”, Republic of Korea.
Keywords: Neurotrophin-4, In vitro maturation, ERK1/2

TOPIC: EPITHELIAL_SKIN

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POSTMORTEM HUMAN DURA MATER CELLS EXHIBIT PHENOTYPIC, TRANSCRIPTOMIC AND GENETIC ABNORMALITIES THAT IMPACT THEIR USE FOR DISEASE MODELING

Argouarch, Andrea R. - *Neurology, University of California, San Francisco, CA, USA*
Schultz, Nina - *Clinical Memory Research, Lund University, Lund, Sweden*
Yang, Andrew - *Neurology, University of California, San Francisco, CA, USA*
Jang, Yeongjun - *Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA*
Garcia, Kristle - *Neurology, University of California, San Francisco, CA, USA*
Cosme, Celica - *Neurology, University of California, San Francisco, CA, USA*
Corrales, Christian - *School of Medicine, University of California, Los Angeles, CA, USA*
Nana, Alissa - *Neurology, University of California, San Francisco, CA, USA*
Karydas, Anna - *Neurology, University of California, San Francisco, CA, USA*
Spina, Salvatore - *Neurology, University of California, San Francisco, CA, USA*
Grinberg, Lea - *Neurology, University of California, San Francisco, CA, USA*
Miller, Bruce - *Neurology, University of California, San Francisco, CA, USA*
Wyss-Coray, Tony - *Neurology and Neurological Sciences, Stanford, CA, USA*
Abyzov, Alexej - *Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA*
Goodarzi, Hani - *Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Seeley, William - *Neurology, University of California, San Francisco, CA, USA*
Kao, Aimee - *Neurology, University of California, San Francisco, CA, USA*

Abstract: Patient-derived cells hold great promise for precision medicine approaches in human health. Human dermal fibroblasts have been a major source of cells for reprogramming and differentiating into specific cell types for disease modeling. Postmortem human dura mater has been suggested as a primary source of fibroblasts for in vitro modeling of neurodegenerative diseases. Although fibroblast-like cells from human and mouse dura mater have been previously described, their utility for reprogramming and direct differentiation protocols has not been fully established. In this study, cells derived from postmortem dura mater are directly compared to those from dermal biopsies of living subjects.

In two instances, we have isolated and compared dermal and dural cell lines from the same subject. Notably, striking differences were observed between cells of dermal and dural origin. Compared to dermal fibroblasts, postmortem dura mater-derived cells demonstrated different morphology, slower growth rates, and a higher rate of karyotype abnormality. Dura mater-derived cells also failed to express fibroblast protein markers. When dermal fibroblasts and dura mater-derived cells from the same subject were compared, they exhibited highly divergent gene expression profiles that suggest dura mater cells originated from a mixed mural lineage. Given their postmortem origin, somatic mutation signatures of dura mater-derived cells were assessed and suggest defective DNA damage repair. This study argues for rigorous karyotyping of postmortem derived cell lines and highlights limitations of postmortem human dura mater-derived cells for modeling normal biology or disease-associated pathobiology.

Keywords: human dura mater, postmortem tissue, neurodegenerative disease

TOPIC: GERMLINE

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BUILDING A NONHUMAN PRIMATE MODEL FOR FETAL OVARIAN DEVELOPMENT

Wamaitha, Sissy E. - *Molecular Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Clark, Amander - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Hsu, Fei-man - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*

Abstract: Although assisted reproductive technologies such as in vitro fertilization provide alternative options for those with reduced fertility, they rely on the ability to produce one's own functional gametes. Where this is not feasible, such as for those with primary ovarian insufficiency or following loss of the oocyte reserve due to chemotherapy, one theoretical approach could involve in vitro gametogenesis (IVG) - differentiation of patient-derived induced pluripotent stem cells (iPSCs) into oocyte-like cells. Regenerating cell types from iPSCs requires emulating the principles of developmental biology to achieve successful lineage specification. For ovarian development, crucial steps occur during the prenatal window, which is almost inaccessible for study in humans. To address this, we use the rhesus macaque as a nonhuman primate cognate, employing a time-mated breeding schedule to acquire fetal samples in the first, second and third trimesters of prenatal life. We collect and analyze single cell transcriptomes to profile the transition from primordial germ cells (the first germline cells to be specified in the embryo) to oocytes within the developing fetal ovary. In tandem we interrogate the identity and fate trajectories of corresponding somatic cell types to investigate the ovarian niche, and explore the origins of primate granulosa and stromal cells. We aim to generate a reference standard against which to evaluate in vitro differentiated germline and somatic cell types, as well identifying prospective regulatory networks and signaling pathways to underpin future IVG technologies.

Keywords: Germ cells, Fetal ovary, Lineage specification

TOPIC: KIDNEY

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TRACING THE ORIGIN OF THE HUMAN AMNIOTIC FLUID STEM CELLS TO THE DEVELOPING FETAL KIDNEY

Gerli, Mattia Francesco Maria - *Division of Surgery and Interventional Science, University College London, UK*
Beesley, Max - *Great Ormond Street Institute of Child Health, University College London, UK*
Martini, Paolo - *Molecular and Translational Medicine, University of Brescia, Italy*
Pellegrini, Marco - *Great Ormond Street Institute for Child Health, University College London, UK*
Panariello, Francesco - *Armenise/Harvard Laboratory of Integrative Genomics, Telethon Institute of Genetics and Medicine, Pozzuoli, Italy*
Xenakis, Theodoros - *Great Ormond Street Institute of Child Health, University College London, UK*
Kunpalin, Yada - *Great Ormond Street Insitute of Child Health, University College London, UK*
Di Biagio, Daniele - *Great Ormond Street Institute of Child Health, University College London, UK*
Subramanian, Sindhu - *Great Ormond Street Institute of Child Health, University College London, UK*
Calà, Giuseppe - *Division of Surgery and Interventional Science, University College London, UK*
Stewart, Benjamin - *Department of Medicine, University of Cambridge, UK*
Jafree, Daniyal - *Great Ormond Street Institute of Child Health, University College London, UK*
Long, David - *Great Ormond Street Institute of Child Health, University College London, UK*
Cacchiarelli, Davide - *Armenise/Harvard Laboratory of Integrative Genomics, Telethon Institute For Genetics and Medicine, Pozzuoli, Italy*
David, Anna - *EGA Institute for Women's Health, University College London, UK*
De Coppi, Paolo - *Great Ormond Street Institute of Child Health, University College London, UK*

Abstract: It is well established that the human amniotic fluid (AF) contains stem and progenitor cells. Amniotic fluid stem cells (AFSC) are mesenchymal, autologous to the fetus, and can be safely isolated during pregnancy. AFSCs have great therapeutic potential in fetal/neonatal regenerative medicine, with many breakthroughs reported since their discovery. However, there is a lack of consensus on the origin and identity of these cells, which hinders their full clinical potential. Through the application of bulk RNA sequencing, our group compared the expression profile of the mesenchymal AFSCs (n=8) to that of mesenchymal stromal cells (MSCs) derived from a range of human fetal tissues. This has demonstrated that the AFSCs show the greatest gene expression similarity to fetal kidney MSCs, suggesting the renal origin of these cells. Furthermore, we found kidney MSCs from later stage fetuses to better resemble the AFSCs' transcriptome. This was also confirmed by multiple different in vitro assays, in which we investigated the presence of developmental renal markers in the AFSCs. By overlaying our bulk RNAseq expression profiles with a single-cell map of the developing kidney, we have then been able to indicate the Metanephric/Cap Mesenchyme as the possible AFSCs' sites of origin. These results indicate that renal cells are transported to the AF through the fetal urine. Therefore,

our current model suggests AFSCs are generated when the fetal kidney epithelial cells exfoliate into the AF, undergoing Epithelial-to-Mesenchymal Transition (EMT). This model is further supported by the co-expression of epithelial and mesenchymal markers, as well as EMT-related genes in the AFSCs. This transition is likely initiated by changes in environmental cues, such as a lack of nutrients and cellular adhesion. Thanks to this dataset, we are currently testing novel markers to improve AFSC prospective isolation and investigating novel avenues for the clinical translation of these cells. Overall, this provides new insights on the different developmental processes involved in the generation of AFSCs, while also significantly improving our understanding of their origin and identity.

Funding Source: Our work is supported by an EC H2020 Marie Skłowdoska-Curie Individual Fellowship, NIHR GOSH BRC, GOSH Charity, UCL GOSICH Institute Development Fund, UCL Global Engagement Fund and by the Wellcome Trust ISSF.
Keywords: Amniotic Fluid Stem Cells, AFSC, fetal stem cells

TOPIC: LIVER

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INVESTIGATING THE IMPACT OF TRISOMY 21 ON XENOBIOTIC BIOTRANSFORMATION USING INDUCED PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS

Marentette, John O. - *School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Roede, James - *School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: Individuals with Down syndrome (DS, Trisomy 21) experience an array of comorbidities in addition to intellectual disabilities including type 1 diabetes, hypothyroidism, obesity, leukemia, inflammation and Alzheimer's disease. These comorbidities are of great relevance to this study due to the fact that genetics, disease states, and cofactor levels can greatly impact xenobiotic biotransformation. To investigate the impact of DS on xenobiotic biotransformation, we utilized hepatocyte-like cells (HLCs) derived from two isogenic induced pluripotent stem cell lines (iPSCs). These cell lines, C3 (CTRL) and C2 (DS) are ideally suited to investigate the impact of trisomy 21 on drug metabolism as they are derived from the same fibroblast cell line, AG06872; therefore, any differences observed from this study are exclusively due to the trisomy of chromosome 21. HLC phenotype was assessed by gene expression (i.e. HNF4α), glycogen synthesis and cytochrome P450 (CYP) activity. We then administered agonists for xenosensor transcription factors, like the aryl hydrocarbon receptor (AhR) and constitutive androgen receptor (CAR), and analyzed downstream drug metabolizing enzyme (DME) expression. HLCs were treated with benzo[a]pyrene (BaP, AhR agonist) for 24 hours to induce downstream Phase 1 DME (ALDH3A1, CYP1A1, CYP1B1) and Phase 2 DME (GSTA1) expression. Folowing BaP (10μM) stimulation, we detected variations in DME gene expression in DS compared to CTRL with ALDH3A1 (1 to 1.9 fold change in CTRL vs DS), CYP1A1 (6.0 to 5.4), CYP1B1 (1.6 to 2.0) and GSTA1 (1.3 to 1.8). To induce downstream CAR gene expression, HLCs were treated with 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)oxime (CITCO, CAR agonist) for 24h and Phase 1 DME (CYP3A4, CYP2B6) expression determined. Following CITCO (1μM) treatment, we saw a drastic increase in CYP3A4 in DS compared to CTRL (1.19 to 11.9 in CTRL vs DS). These studies highlight the inherent differences that trisomy 21 has on xenobiotic biotransformation and induction of Phase 1 and 2 DME. Further, these data show that DS individuals do not metabolize xenobiotics in a manner similar to euploid, indicating that additional characterizations are necessary to effectively understand drug and toxicant metabolism in this unique genetic background.

Keywords: Hepatocyte, Stem Cell, Xenobiotic Biotransformation

TOPIC: MUSCULOSKELETAL

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AN ENGINEERED MULTICELLULAR STEM CELL NICHE FOR THE 3D DERIVATION OF HUMAN MYOGENIC PROGENITORS FROM IPSCS

Mashinchian, Omid - *Cell Biology, Nestlé Institute of Health Science, Nestlé Research, Lausanne, Switzerland*

Abstract: Fate decisions in the embryo are controlled by a plethora of microenvironmental interactions in a three-dimensional niche. To investigate whether aspects of this microenvironmental complexity can be engineered to direct myogenic human induced pluripotent stem cell (hiPSC) differentiation, we screened cell types present in the developmental or adult stem cell niche in heterotypic suspension embryoids. We identify the combination of embryonic endothelial cells and fibroblasts as highly permissive for myogenic specification of hiPSCs. Combined with Wnt and FGF pathway mediated lineage-priming, embryoid differentiation allows for the isolation of >99% pure Pax7 positive skeletal muscle progenitors within a two-week time-window. Myogenic differentiation of hiPSCs in heterotypic embryoids relies on a specialized structural microenvironment and activates the MAPK, PI3K/AKT and Notch pathways. Embryoid derived myogenic cells reactivate after repeated injury and, compared to adult human myoblasts, display enhanced fusion to muscle fibers and lead to stronger muscles when transplanted into a mouse model of Duchenne muscular dystrophy. Altogether, we provide a highly efficient and scalable suspension-based protocol for 3D derivation of Pax7 positive myogenic progenitors from hiPSCs.

Keywords: Embryoids & organoids, Skeletal muscle development, Human induced pluripotent stem cell (hiPSC)

TOPIC: NEURAL

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STEM CELL MODEL OF SPINAL CORD DEVELOPMENT GENERATES LARGE NUMBERS OF BONA-FIDE SENSORY INTERNEURONS

Gupta, Sandeep - *Neurobiology, University of California, Los Angeles, CA, USA*
Kawaguchi, Riki - *Semel Institute of Neuroscience and Human behavior, University of California, Los Angeles, CA, USA*
Heinrichs, Eric - *Department of Human Genetics, University of California, Los Angeles, CA, USA*
Mandric, Igor - *Henry Samueli School of Engineering and Applied Sciences, University of California, Los Angeles, CA, USA*
Castellanos, Stephanie - *Department of Biology, California State University, Northridge, Los Angeles, CA, USA*
Gallardo, Selena - *Department of Neurobiology, UCLA, Los*

Angeles, CA, USA
Novitch, Bennett - *Department of Neurobiology, UCLA, Los Angeles, CA, USA*
Butler, Samantha - *Department of Neurobiology and Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, USA*

Abstract: Restoring sensation after injury or disease requires a reproducible method of generating large quantities of bona fide somatosensory interneurons. These interneurons arise in the dorsal spinal cord and are specific for different sensory modalities. Here, we reproduced the logic of dorsal spinal cord development using two growth factors, retinoic acid (RA) and bone morphogenetic protein 4 (BMP4), and obtained the entire complement of dorsal interneurons (dI1-dI6) from both mouse and human embryonic stem cells (ESCs). Bioinformatic approaches, comparing in vivo and in vitro datasets, have revealed that these ESC-derived dIs are strikingly similar to their endogenous counterparts, with maturation signatures that include the neuropeptides that regulate the sensory processing of pain, itch, and temperature. Novel signatures were also identified, including distinct psychoactive and analgesic drug-related signaling pathways in different dI populations, opening new horizons for drug testing platforms to identify new analgesic compounds. This in vitro recapitulation of dorsal spinal cord development has also permitted new insights into unresolved developmental mechanisms. First, we have unraveled the hierarchical nature of dI fate specification mediated by RA and BMP4 signaling, which determines dI diversity. Second, we demonstrate that BMP4 induces Wnt signaling to act as a potent mitogen, rather than a morphogen, to regulate dorsal progenitor proliferation. Chemically activating Wnt signaling using CHIR permits the dramatic expansion of ESC-derived dorsal progenitor cultures, which can readily differentiate into diverse populations of dIs upon CHIR removal. Together, these studies identify the conditions to mass-produce bona fide sensory interneurons required for many clinical applications, such as restorative spinal cord transplantations, drug testing platforms, and sensory disease modeling.

Keywords: spinal sensory interneurons, mouse and human stem cells, Wnt signaling

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CELL IDENTITY LOGIC UNDERLYING DIRECT NEURONAL CONVERSION BY MICRORNAS

Cates, Kitra - *Department of Developmental Biology, Washington University, Saint Louis, MO, USA*

Abstract: The ability to alter the cell fate between different human somatic cell types offers novel experimental means to study cells that are not readily attainable, such as neurons. One example is the direct conversion of human dermal fibroblasts (hDF) into microRNA-induced neurons (miNs) by the ectopic expression of microRNAs-9/9* and -124 (miR-9/9*-124). These neurogenic microRNAs instruct extensive restructuring across the somatic cell chromatin landscape, driving non-neuronal chromatin loci closure and pan-neuronal loci accessibility. miR-9/9*-124 orchestrate a stepwise fate conversion on the transcriptional level as well, starting with fibroblast gene program silencing, followed by neuronal gene program activation. This direct conversion provides a platform to study the epigenetic mechanisms underlying cell fate erasure and the plasticity of cell fate memory. We have identified the chromatin architecture and gene network erasure across non-neuronal loci in hDF; however, it remains unknown what master gene networks regulate non-neuronal cell identity. To understand somatic cell fate erasure, we converted multiple non-neuronal cell lineages derived from the human brain into miNs, including dura fibroblasts (hDuF), astrocytes (hAst), brain vascular pericytes (hBVP), and brain vascular smooth muscle cells (hBVSMC). miR-9/9*-124 are sufficient to convert each cell line into miNs, robustly inducing neuronal gene expression and morphology across lineages. We completed transcriptome sequencing analysis during early reprogramming across hDF, hDuF, hAst, hBVP, and hBVSMC expressing miR-9/9*-124 or a control, non-specific microRNA. This dataset reveals the transcriptional logic of fate erasure and begins to distinguish the cellular plasticity of miR-9/9*-124 reprogramming across cells from multiple developmental lineages. Our data expands existing reprogramming paradigms to alternate somatic cells and elucidates methods to leverage cell fate memory for regenerative medicine research.

Keywords: reprogramming, fate plasticity, microRNAs

TOPIC: PANCREAS

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IDENTIFYING INSULIN-SECRETING β-CELL ENHANCERS THROUGH ENHANCER MAPPING AND LARGE-SCALE CRISPRI-MEDIATED PERTURBATION SCREENS

Zhang, Nan - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Pulecio, Julian - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Luo, Renhe - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Murphy, Dylan - *Sanford I Weill Department of Medicine, Weill Cornell Medicine, New York, NY, USA*
Wong, Wilfred - *Computational and Systems Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Leslie, Christina - *Computational and Systems Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Apostolou, Effie - *Sanford I Weill Department of Medicine, Weill Cornell Medicine, New York, NY, USA*
Huangfu, Danwei - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Abstract: A major component of diabetes is the loss of functional β cells that secret insulin to regulate blood glucose homeostasis. To uncover diabetes risk factors, it is important to first understand the developmental control of β cells. Indeed, many studies have been carried out to identify and interrogate protein-coding regulators of β-cell development and function. Yet, the role of non-coding regulatory elements involved in the establishment and maintenance of functional β cells remain inexplicit. To fill this gap, in this study, I aim to identify functional enhancers that control β-cell development. First, I plan to construct three-dimensional (3D) enhancer networks in different developmental stages using human pluripotent stem cells (hPSCs)-based β cell differentiation. So far, our lab has generated pilot Hi-C maps on cells from undifferentiated hPSC, definitive endoderm (DE) and primitive PDX1+NKX6.1– pancreatic progenitor (PP1) stages. Interestingly, we observed increased distal interactions during pancreatic differentiation at several β-cell developmental gene loci (e.g., PDX1, INS and MAFB). By further increasing the sequencing depth and generating 3D enhancer networks at later differentiation stages, I expect to uncover more regulatory interactions associated with β-cell development in the future. Next, I plan to dissect the role of

putative enhancers identified in the previous step through large-scale functional interrogations. Our lab has developed two CRISPR-based screening approaches: a gene-centric approach and a cell-state centric approach. By applying these two screening approaches, we have successfully identified enhancers for several pancreatic differentiation-related genes (e.g. PDX1, Sox17, EOMES and GATA6). Later, I will extend the screening approach and further interrogate enhancers mapped with 3D genomic datasets through large-scale CRISPR interference-mediated perturbation screens using hPSC-β cells. Overall, results from this study will help us to understand how enhancers are involved in β-cell differentiation and provide mechanistic insights into the crosstalk between 3D chromatin organization and transcriptional regulation.

Funding Source: 1U01DK128852-01, NIH/NIDDK
Keywords: β cell differentiation, 3D enhancer network, large-scale CRISPRi screen

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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DERIVATION OF HUMAN INDUCED TROPHOBLAST STEM CELLS VIA MRNA-MEDIATED REPROGRAMMING OF FIBROBLASTS

Tan, Jia Ping - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Zhou, Yichen - *Department of Microbiology, Monash University, Clayton, Australia*
Mohenska, Monika - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Sun, Guizhi - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Chen, Joseph - *Australian Regenerative Medicine Institute, Monash University, Clayton, Australia*
Sun, Yu Bo Yang - *Australian Regenerative Medicine Institute, Monash University, Clayton, Australia*
Nie, Guiying - *STEM|Health and Biomedical Sciences, RMIT University, Bundoora, Australia*
Liu, Xiaodong - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*
Polo, Jose - *Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia*

Abstract: The human trophectoderm gives rise to the trophoblast cell derivatives that are critical in mediating cross-talk between the fetal-maternal interface. When captured in vitro, cells of the trophectoderm lineage give rise to self-renewal trophoblast stem cells (TSCs). Recently, we have established that human induced trophoblast stem cells (iTSCs) could be derived from fibroblasts via OCT4, KLF4, SOX2 and c-MYC (OKSM)-mediated reprogramming that are delivered by non-integrative Sendai virus. Here, we present an alternative mRNA-mediated reprogramming approach for human iTSCs derivation. The mRNA-derived iTSCs demonstrate long-term proliferative potential, express key trophoblast markers and trophoblast lineage-associated C19 miRNAs. Transcriptional profiling shows that the mRNA-derived iTSCs are similar to the placenta-derived TSCs, and they are also capable of functionally differentiating into human chorionic gonadotropin (hCG)-secreting syncytiotrophoblasts and HLA-G-expressing extravillous cytotrophoblasts. Together, this approach represents a transgene-free system for robust derivation of iTSCs which could be useful in modelling placental biology and diseases.

Keywords: induced trophoblast stem cells, somatic reprogramming, Placenta

TOPIC: PLURIPOTENT STEM CELLS

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USING THE EPITHELIAL TO MESENCHYMAL TRANSITION IN HIPSCS AS A MODEL TO STUDY CELL STATES AND STATE TRANSITIONS BY COMBINING CELL BEHAVIOR, CELL ORGANIZATION, AND CELL IDENTITY

Dalgin, Gokhan - *Allen Institute for Cell Science, Allen Institute for Cell Science, Seattle, WA, USA*
Gunawardane, Ruwanthi - *Allen Institute for Cell Science, Allen Institute for Cell Science, Seattle, WA, USA*

Abstract: The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. The epithelial to mesenchymal transition (EMT) is a state change that occurs during development, wound healing, cancer metastasis, and fibrosis. We use 3D, live-cell imaging across several spatial scales to capture dynamic cell behavior, including shape, migration analysis, and organelle localization in a model of EMT induced in hiPSCs . We are integrating 3D live-cell imaging with fixed-cell immunolabeling and scRNAseq-informed RNA-FISH to understand the relationship between cell behavior, organization and cell fate. Based on these data, we will determine whether cells in the same population take different trajectories, the same trajectory with different timing, or undeterminable trajectories. We are now generating different conditions of EMT induced in hiPSCs to test whether the relationships between cell organization, behavior, and fate that we establish are generalizable or depend on the specific model of EMT. We propose that this multi-modal, multi-scale approach can serve as a framework for studying EMT in many contexts and in understanding cell states and transitions more generally. Detailed information about our cell lines can be found at www.allencell.org

Keywords: EMT, cell states, live-cell imaging

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THE WNT/TCF7L1 TRANSCRIPTIONAL REPRESSOR AXIS DRIVES PRIMITIVE ENDODERM FORMATION BY ANTAGONIZING NAIVE AND FORMATIVE PLURIPOTENCY

Athanasouli, Paraskevi - *Development and Regeneration, Katholieke Universiteit Leuven, Belgium*
Balli, Martina - *Department of Development and Regeneration, KU Leuven, Belgium*
De Jaime-Soguero, Anchel - *Department of Development and Regeneration, KU Leuven, Belgium*
Boel, Annekatrien - *Department of Reproductive Medicine, Ghent University, Ghent, Belgium*
Papanikolaou, Sofia - *Department of Rheumatology, University of Crete, Heraklion, Greece*
Van der Veer, Bernard - *Department of Development and*

Regeneration, KU Leuven, Belgium
Janiszewski, Adrian - *Department of Development and Regeneration, KU Leuven, Belgium*
Lo Nigro, Antonio - *Department of Development and Regeneration, KU Leuven, Belgium*
Aulicino, Francesco - *Centre for Genomic Regulation, Barcelona, Spain*
Koh, Kian Peng - *Department of Development and Regeneration, KU Leuven, Belgium*
Pasque, Vincent - *Department of Development and Regeneration, KU Leuven, Belgium*
Cosma, Maria Pia - *Centre for Genomic Regulation, Barcelona, Spain*
Verfaille, Catherine - *Department of Development and Regeneration, KU Leuven, Belgium*
Heindryckx, Bjorn - *Department of Reproductive Medicine, Ghent University, Ghent, Belgium*
Nikolaou, Christoforos - *Biomedical Sciences Research Center "Alexander Fleming", Institute of Bioinnovation, Athens, Greece*
Lluis, Frederic - *Department of Development and Regeneration, KU Leuven, Belgium*

Abstract: Early during preimplantation development and in heterogeneous mouse embryonic stem cells (mESC) culture, pluripotent cells are specified towards either the primed epiblast (EPI) or primitive endoderm (PE) lineage. Canonical Wnt signaling is crucial for safeguarding naive pluripotency and embryo implantation, yet the role and relevance of canonical Wnt inhibition during early mammalian development remains unknown. Here, we use Wnt-inhibiting culture conditions and loss-/gain-of-function approaches to determine the role of the Wnt pathway and the TCF/LEF factors in the context of the EPI/PE binary cell fate decision. We demonstrate that Wnt/TCF7L1 transcriptional repression promotes PE differentiation of mESCs and in mouse preimplantation inner cell mass (ICM). Time-series RNA sequencing (RNA-seq) and promoter occupancy data reveal that TCF7L1 binds and represses not only genes encoding essential naive pluripotency factors but also indispensable regulators of the formative pluripotency program, including Otx2 and Lef1. Consequently, TCF7L1 promotes pluripotency exit and suppresses epiblast lineage formation, thereby forcing cells into PE specification. On the contrary, deletion of Tcf7l1 severely abrogates PE differentiation without restraining epiblast priming. Taken together our study underscores the importance of transcriptional Wnt inhibition in regulating lineage segregation in ESCs and preimplantation embryo development as well as identifies TCF7L1 as key regulator of this process.

Keywords: Embryonic stem cells, Wnt/b-catening signaling, Primitive endoderm specification

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SIRT2 AS A KEY REGULATOR CONTROLLING MITOCHONDRIAL DYNAMICS AND METABOLIC REPROGRAMMING DURING HUMAN SOMATIC CELL REPROGRAMMING

Cha, Young - *Department of Psychiatry, McLean Hospital/ Harvard Medical School, Belmont, MA, USA*
Kim, Taewoo - *Department of Psychiatry, McLean Hospital/ Harvard Medical School, Belmont, MA, USA*
Leblanc, Pierre - *Department of Psychiatry, McLean Hospital/ Harvard Medical School, Belmont, MA, USA*
Cohen, Bruce - *Department of Psychiatry, McLean Hospital/ Harvard Medical School, Belmont, MA, USA*
Kim, Kwang-Soo - *Department of Psychiatry, McLean Hospital/ Harvard Medical School, Belmont, MA, USA*

Abstract: Metabolic reprogramming (known as "Warburg effect") from mitochondrial oxidative phosphorylation toward glycolysis is critical for not only determining pluripotent stem cell fate and function but also inducing pluripotency from terminally differentiated somatic cells. Despite its importance, our understanding of the molecular mechanisms underlying mitochondrial metabolic reprogramming is incomplete. Notably, SIRT2 downregulation is a hallmark of pluripotent stem cells as well as somatic cell reprogramming into a pluripotent state. Here, we investigated specific roles in mitochondrial dynamics that are coordinated by SIRT2 and its potential downstream targets and pathways. In particular, we report that SIRT2 regulates mitochondrial dynamics and oxidative metabolism via a DRP1-dependent manner. In addition, we explored how SIRT2 link mitochondrial remodeling and oxidative phosphorylation to the somatic reprogramming process. We will discuss our novel findings regarding the potential functional roles of SIRT2 as a key regulator of mitochondrial remodeling and metabolic reprogramming during the human somatic cell reprogramming process.

Funding Source: This work was supported by NIH grants (NS070577 and OD024622) and the Parkinson's Cell Therapy Research Fund at McLean Hospital.
Keywords: Human somatic cell reprogramming, Mitochondrial remodeling, SIRT2

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NAÏVE AND PRIMED HUMAN PLURIPOTENT STEM CELLS RELY ON TGFβ SIGNALLING TO MAINTAIN PLURIPOTENCY

Osnato, Anna - *Wellcome - MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Brown, Stephanie - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Krueger, Christel - *Bioinformatics Group, The Babraham Institute, Cambridge, UK*
Grey-Wilson, Charlotte - *Wellcome–MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Andrews, Simon - *Bioinformatics Group, The Babraham Institute, Cambridge, UK*
Collier, Amanda - *Epigenetics Programme, The Babraham Institute, Cambridge, UK*
Nakanoh, Shota - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Quiroga Londoño, Mariana - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Wesley, Brandon - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Muraro, Daniele - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Brumm, Sophie - *Human Embryo and Stem Cell Laboratory, The Francis Crick Institute, London, UK*
Niakan, Kathy - *Human Embryo and Stem Cell Laboratory, The Francis Crick Institute, London, UK*
Ortmann, Daniel - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*
Rugg-Gunn, Peter - *Epigenetics Programme, The Babraham Institute, Cambridge, UK*
Vallier, Ludovic - *Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, UK*

Abstract: Human pluripotent stem cells (hPSCs) are grown in vitro as two broadly different states termed naïve and primed. The two states diverge in their embryonic identity with naïve hPSCs resembling pluripotent cells of pre-implantation embryos and primed hPSCs equivalent to the post-implantation epiblast. The signalling pathways that maintain primed hPSCs have been well characterised, revealing a critical role for TGFβ/Activin/Nodal signalling. In contrast, the signalling requirements of naïve human pluripotency have not been fully established. Here, we demonstrate that TGFβ signalling is active and necessary to maintain naïve hPSCs. The downstream effector proteins – SMAD2/3 – bind common sites in naïve and primed hPSCs, including shared pluripotency genes. In naïve hPSCs, SMAD2/3 additionally bind to active regulatory regions near to naïve pluripotency genes. Inhibiting TGFβ signalling in naïve hPSCs causes the downregulation of SMAD2/3-target genes and exit from pluripotency. Single-cell analyses reveal that naïve and primed hPSCs follow different transcriptional trajectories after inhibition of TGFβ signalling. Primed hPSCs differentiate heterogeneously into the ectoderm lineage, generating neural, neural crest and epidermal progenitors. Naïve hPSCs instead transition into trophectoderm and have the potential to differentiate into its derivatives such as cytotrophoblast, extravillous trophoblast and syncytiotrophoblast. These results establish that there is a continuum for TGFβ pathway function in human pluripotency spanning a developmental window from naïve to primed states, with a central role in protecting hPSCs against differentiation.

Funding Source: The work was mainly supported by the ERC (AO, LV, DO), and also the BBSRC and the MRC (PJR-G).
Keywords: TGFβ, hESCs, Pluripotency

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MARSUPIAL IPSCS PROVIDE INSIGHTS INTO EVOLUTIONARY ORIGINS OF PLURIPOTENCY NETWORKS

Hutchinson, Ashlee - *BioSciences, University of Melbourne, Murrumbeena, Australia*
Frankenberg, Stephen - *BioSciences, The University of Melbourne, Australia*
Gallego Romero, Irene - *BioSciences, The University of Melbourne, Australia*
Hoblos, Hanadi - *BioSciences, The University of Melbourne, Australia*
Ibeh, Neke - *BioSciences and St. Vincent's Institute for Medical Research (SVI), The University of Melbourne, Australia*
Pask, Andrew - *BioSciences, The University of Melbourne, Australia*

Abstract: Marsupials diverged from eutherian mammals 160 million years ago and have much to teach us about fundamental mammalian biology. We can use this evolutionary divergence to define conserved pathways that regulate developmental processes and to better understand mammalian pluripotency networks. However, resources that are well established for conventional laboratory models are underdeveloped for marsupials. For instance, the absence of an inner cell mass in marsupial embryos impedes the easy isolation of embryonic stem cells. Therefore we focused on the generation of induced pluripotent stem cells (iPSCs), using an Australian carnivorous marsupial amenable to laboratory husbandry, the fat-tailed dunnart (Sminthopsis crassicaudata). We report the first successful derivation of dunnart iPSCs, with a refined protocol for reprogramming dunnart somatic cells to a pluripotent state. This protocol has now been used to produce two distinct lines and multiple clones. We confirm pluripotency using data from RT-qPCR, immunocytochemistry, and single-cell RNA sequencing. In addition to the core pluripotency genes and surface antigens, dunnart iPSCs express additional markers for pluripotency including the marsupial POU5F1 orthologue, POU5F3, that can be used as a basis for comparison with other model species. Dunnart iPSCs produce embryoid bodies and these differentiate into cell-type representatives for endoderm, mesoderm and ectoderm. Furthermore, dunnart iPSCs are capable of differentiation to specific cell lineages, producing cardiomyocyte-like cells using human protocols. These cell lines make the dunnart a tractable model, enabling direct comparison of iPSC gene expression and implicated reprogramming pathways, with those for eutherian models. This will facilitate a richer understanding of pluripotency, early embryonic development and cell-lineage specification in marsupials, and the fundamental pathways that mediate these events across all mammal lineages. Finally, defined protocols for the derivation of marsupial stem cells have huge implications for conservation efforts and will enable the preservation of genetic diversity of Australia's vulnerable mammals.

Funding Source: This work was supported by Discovery Project funding (DP160103683) from the Australian Research Council to Andrew Pask
Keywords: Induced Pluripotent Stem Cells (iPSCs), Mammalian Pluripotency, Marsupial

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LINEAGE ANALYSIS OF CELL STATES DETERMINING REPROGRAMMING INTO INDUCED PLURIPOTENT STEM CELLS

Jain, Naveen - *Department of Genetics, University of Pennsylvania, Philadelphia, PA, USA*
Goyal, Yogesh - *Department of Cell and Developmental Biology, Northwestern University, Chicago, IL, USA*
Emert, Benjamin - *California Institute of Technology, Department of Bioengineering and Applied Physics, Pasadena, CA, USA*
Mellis, Ian - *Department of Pathology, Columbia University, New York, NY, USA*
Dunagin, Margaret - *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Cote, Christopher - *Department of Molecular Plant Biology, Washington State University, Pullman, WA, USA*
Reffsin, Sam - *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Dardani, Ian - *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*
Raj, Arjun - *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA*

Abstract: 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 cell identity 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 what if anything is different about the rare cells that become iPSCs and when their ability to reprogram successfully is established. Here, we show that "primed" cell 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 "Rewind". We show that Rewind can effectively distinguish between primed and unprimed cells before OKSM exposure via unique and transcribed DNA barcodes. We also show candidate gene expression markers of the primed states identified by Rewind. These markers are significantly upregulated in primed cells that eventually become iPSCs and are associated with proliferation, transcription, and plasticity. 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.

Funding Source: Penn MSTP (T32GM717044), NIH/NICHD (F30 HD103378), Michael Brown Fellowship
Keywords: reprogramming, barcoding, single-cell

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GENERATION OF ATRIAL ENGINEERED HEART TISSUES FROM HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ATRIAL CARDIOMYOCYTES

Qadan, Maha A. - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Qadan, Maha - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Gore-Panter, Shamone - *Cardiovascular and Metabolic Sciences and Biological, Geological and Environmental Sciences, Lerner Research Institute of the Cleveland Clinic Foundation and Cleveland State University, Cleveland, OH, USA*
Lovano, Beth - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Castel, Laurie - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Rennison, Julie - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Chung, Mina - *Cardiovascular and Metabolic Sciences and Cardiovascular Medicine, Heart & Vascular Institute, Lerner Research Institute of the Cleveland Clinic Foundation, Cleveland, OH, USA*
Van Wagoner, David - *Cardiovascular and Metabolic Sciences, Lerner Research Institute of the Cleveland Clinic Foundation and Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA*

Abstract: Atrial cardiomyocytes (aCMs) differentiated in monolayers from human iPSCs are structurally and functionally immature, restricting their utility for disease modeling and drug testing for cardiac arrhythmias such as atrial fibrillation (AF). Generation of 3D engineered heart tissues (EHTs) that recapitulate adult atrial tissue in structure and complexity remains a challenge. We have established an aEHT-based arrhythmia model using patient-specific iPSCs generated by reprogramming PBMCs of AF patients. The impact of culture substrates on maintaining iPSCs' pluripotency and derivation of aCMs for generation of chamber-specific aEHT models remains elusive. We hypothesized that substrates used to maintain iPSC cultures and support aCM differentiation play a role in efficient generation and maturation of aEHTs. We tested the impact of Matrigel, Geltrex, and rhVitronectin in 21% or 5% O2. aEHTs were generated by combining iPSC-aCMs with a fibrin-based hydrogel suspended between flexible silicon posts. Derivation of aCMs was efficient, with 70%-95% of cells positive for cardiac troponin-T, expressing atrial-specific markers MLC2A and PITX2c, and lacking expression of the ventricular-specific marker MLC2V. The response of iPSCs and aCMs to specific substrates was cell line dependent and varied by sex, passage, and incubator pO2. Interestingly, efficient generation of aEHTs from iPSC-aCMs was independent of the substrate used for maintaining iPSCs and aCM differentiation. aEHTs maintain spontaneous beating. Electron microscope studies show that sarcomeric structure of aEHTs is more similar to adult atrial tissues than that of aCM monolayers. Initial transcriptomic assessments of aEHTs indicated that metabolic pathways are also much closer to adult left atrial tissues than to aCMs in monolayers. The aEHTs format provides mechanical cues that, with time, promote metabolic and contractile maturation, recapitulating key features of adult tissue. We are conducting additional functional electrophysiology, calcium cycling and contractility studies and using RNA sequencing to decipher the path towards maturation of aEHTs. This model can be used to evaluate patient-specific pathophysiological mechanisms of AF and to test pharmacological interventions tailored to the needs of individual patients.

Funding Source: This work was supported by grants from the National Institutes of Health (R01 HL111314) and the American Heart Association (18SFRN34170442).
Keywords: Human Induced Pluripotent Stem Cells, Atrial Engineered Heart Tissues, Reprogram Peripheral Blood Mononuclear Cells

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EPITHELIAL-MESENCHYMAL TRANSITION AND STEM CELL BIOMARKER IDENTIFICATION IN PATIENT-DERIVED BREAST CANCER 3D CELL MODELS USING A MICROFLUIDICS-BASED SYSTEM

Nikolov, Katya - *Product Development, Protein Fluidics, Inc., Burlingame, CA, USA*
Suzuki, Mahomi - *Life Science Application, Yokogawa Electric Corporation, Kanazawa-shi, Japan*
Brock, Courtney - *Molecular and Cellular Oncology, Tulane University, New Orleans, LA, USA*
Collins-Burow, Bridgette - *Hematology-Oncology, Tulane University, New Orleans, LA, USA*
Burow, Matthew - *Hematology-Oncology, Tulane University, New Orleans, LA, USA*
Cromwell, Evan - *Research and Development, Protein Fluidics, Inc., Burlingame, CA, USA*

Abstract: Physiologically relevant 3D cell models are essential for pre-clinical research and drug discovery. 3D cell cultures better recapitulate the in vivo tissue and tumor microenvironments with regards to cell-cell and cell-matrix interactions. These models offer the possibility to better analyze cancer biology and functionality as compared to 2D cell models. We present a breakthrough

advancement in biomarker staining and detection – combining automated immunofluorescence staining of 3D cell models using the microfluidic-based Pu·MA System with CellVoyager CQ1 System high resolution confocal imaging. In this study we demonstrate the application of this automated workflow to investigate the expression of epithelial-to-mesenchymal transition (EMT) and stem cell markers in patient derived breast cancer models. EMT is strongly implicated in cancer metastatic process and is associated with poor prognosis in breast cancer. CD24 and CD44 cell-surface expression has been proposed as a marker for breast cancer stem cells. CD24−/44+ phenotype has been linked with EMT. In this study we used cellular derivatives of primary TNBC tumors, that were grown into 3D tumoroids. Two patient-derived models with different metastatic potential were evaluated for the expression of epithelial marker E-cadherin, mesenchymal marker Vimentin, F-actin (cytoskeletal component), CD24 and CD44 markers. We further evaluated the viability response of these tumoroids to different drugs (paclitaxel, romidepsin and trametinib), as well as the treatment effect on the expression status of these markers. The workflow consists of 1) creating tumoroids in multicavity low attachment plates, 2)loading tumoroids into a flowchip in hydrogel, 3) exposure of the tumoroids to drugs in the flowchip, 4) automated execution of viability assay and IF staining steps and 5) confocal high-resolution imaging within the flowchip. The workflow presented here has high utility value for studying the interplay between E-cadherin cell adhesion machinery ,Vimentin and associated partners like F-actin, its role in epithelial-to-mesenchymal transition and metastasis, and how they link to cancer stem cells and drug resistance.

Keywords: 3D cell model, breast cancer, biomarker

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DISSECTING FUNCTIONS OF THE OCT4 ENHANCERS BY A LOSS-OF-FUNCTION APPROACH

Schmitz, Daniel - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Sakurai, Masahiro - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Wei, Yulei - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Yu, Leqian - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Wu, Jun - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*

Abstract: At the center of pluripotency is the master transcriptional regulator OCT4 (POU5F1). In pluripotent cells, OCT4 acts in concert with various other factors to establish a transcriptional network necessary for maintaining the pluripotent states. Transcription of OCT4 is driven by two distinct enhancers, distal (DE) and proximal (PE), whose activities dynamically change during early mammalian development. The first pluripotent cell population to arise during development (epiblast cells of the inner cell mass which represent the “naïve” state of pluripotency) utilize the DE to drive OCT4 expression, whereas the last pluripotent cells to exist in development (cells of the peri-gastrulation epiblast which represent the “primed” state of pluripotency) utilize the PE. Why these two different enhancers exist to drive OCT4 expression is unknown. To better understand both the function of and necessity for each enhancer in pluripotency and development, we have taken a loss-of-function approach by removing each enhancer from mouse pluripotent stem cells (PSCs) and zygotes using CRISPR/Cas9. By assaying the pluripotent states and developmental potential of cells lacking each enhancer, we have found that either enhancer is sufficient for in vitro PSC differentiation, though both are needed for proper in vivo development. Naïve mouse embryonic stem cells (mESCs) lacking both copies of the PE (ΔPE-mESCs) are unable to be maintained in the primed state, though still maintain the ability to form teratomas and undergo retinoic acid-mediated differentiation comparable to WT mESCs. Similarly, human embryonic stem cells lacking the DE (ΔDE-hESCs) cannot be maintained in the naïve state but are able to form teratomas and differentiate in vitro similar to WT hESCs. Despite the ability of ΔPE-mESCs to differentiate in vitro, we have not successfully generated mice lacking both copies of the PE, suggesting a necessity for the PE in vivo.

Keywords: Enhancer, OCT4, State

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DECIPHERING TRANSCRIPTION FACTOR LOGIC IN ENHANCER NETWORK IN HUMAN PLURIPOTENT STEM CELLS

Yan, Jielin - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Luo, Renhe - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Huangfu, Danwei - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Abstract: Enhancers regulate gene expression and have a crucial role in enabling the faithful execution of lineage-specific transcriptional programs during development as well as safeguarding against diseases such as cancer and limb malformations. Therefore, the identification and characterization of the enhancers of key developmental regulators are important to improve the understanding of human health. While candidate enhancers can be mapped using bulk-level sequencing of chromatin features predicative of active regulatory elements, their direct connection to the transcriptional output of their target genes is often not established. Global enhancer activity is typically dictated by a handful of lineage-specific transcription factors (TFs), such as OCT4, NANOG, and SOX2 in human pluripotent stem cells (hPSCs). The expression of these TFs is in turn regulated by their own enhancers through the binding of themselves and other such core TFs. However, how the enhancers of these core TFs ensure their proper expression in hPSCs and during differentiation has not been systematically investigated. To address this question, we performed a CRISPR dCas9-KRAB screen in hPSCs to identify pluripotency enhancers. The hits include a novel NANOG enhancer, whose heterozygous deletion reduced NANOG and OCT4 expression in hPSCs and accelerated the downregulation of these two pluripotency markers and the upregulation of lineage-specific markers during differentiations. These results implicate that this enhancer safeguards the robustness of pluripotency. In addition, the heterozygosity of this enhancer might facilitate the disintegration of pluripotency regulatory network and its replacement by that of the succeeding lineage. To further delineate the regulatory relationships between NANOG, OCT4, and other lineage TFs, I will show preliminary work on 1) disrupting TF binding motifs with both targeted and unbiased mutagenesis to understand the TF logic governing the activity of pluripotency enhancers in hPSCs and 2) quantitatively profiling the dynamics of global transcription and enhancer accessibility changes following the perturbation of core TF enhancers using CRISPR and single-cell multi-omic ap-

proach. Taken together, these experiments will help to decipher enhancer network in development modeled by hPSCs.

Keywords: Enhancers, Transcription factors, CRISPR perturbation

TOPIC: EARLY EMBRYO

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COMPARATIVE TRANSCRIPTOME ANALYSIS OF TROPHOBLAST STEM CELLS FROM PORCINE IN VITRO-FERTILIZED AND SOMATIC CELL NUCLEAR-TRANSFERRED EMBRYOS

Kim, Eunhye - *Collage of Veterinary Medicine, Chungbuk National University, Jinju, Korea*
Hyun, Sang-Hwan - *Collage of Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Somatic cell nuclear transfer (SCNT) technique utilized to generate cloned pigs has a low efficiency and fetuses produced by SCNT exhibit limited developmental potential with abnormalities in placenta. Here, we established trophoblast stem cell (TSC) lines from porcine SCNT and in vitro fertilized (IVF) blastocysts (TSC_NT and TSC_IVF lines, respectively) by activation of Wingless/Integrated (Wnt) and EGF and inhibition of TGFb and Rho-associated protein kinase (ROCK) with ascorbic acid supplement. Both of the established porcine TSC lines showed TSC markers such as KRT7, CDX2, and TEAD4. However, TSC_NT lines displayed significantly upregulated mRNA expressions of steroid hormone biosynthesis-related genes such as CYP11A1, HSD3B1 and HSD11B2, compared to those in TSC_IVF lines. Also, we discovered significantly downregulated expressions of key gene at apposition of porcine blastocysts during implantation, such as ITGB6, only in TSC_NT lines. In addition, the expression of YBX2, transcriptional regulators for RNA stability was significantly lower in porcine TSC_NT lines than TSC_IVF lines. Interestingly, the key transcriptional factor for angiopoiesis, VEGFA, was downregulated in TSC_NT lines compared to TSC_IVF lines. These data suggest that our novel method could provide in vitro research tools to study abnormal development and dysfunction of trophoblast-derived placenta in cloned pigs produced by SCNT.

Funding Source: This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korean Government (No. 2021R1C1C2007132).
Keywords: Trophoblast stem cells, Cloned pig, SCNT

TOPIC: EYE AND RETINA

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THE ROLE OF EPIGENETIC REGULATION OF WNT5A IN DIABETIC CORNEAL EPITHELIAL WOUND HEALING AND LIMBAL STEM CELL EXPRESSION

Shah, Ruchi - *Biomedical Sciences/Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Spektor, Tanya - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Punj, Vasu - *Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*
Ding, Hui - *Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Patil, Rameshwar - *Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Turjman, Sue - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ghiam, Sean - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Chun, Steven - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Amador, Cynthia - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Inzalaco, Jake - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Kim, Jiho - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Tolstoff, Sasha - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Yampolsky, Sabina - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Weisenberger, Dan - *Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*
Rabinowitz, Yaron - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Meguen, Ezra - *David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA*
Saghizadeh, Mehrnoosh - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ljubimova, Julia - *Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Kramerov, Andrei - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ljubimov, Alexander - *Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Diabetes mellitus (DM) is the most widespread blinding disease in working-age adults. Up to 70% of diabetic patients show corneal complications with persistent epithelial alterations and impaired wound healing, which may occur due to the dysfunction of limbal epithelial stem cells (LESC). Due to its epigenetic nature, DM involves altered DNA methylation and miRNA changes. Previously, we reported the Wnt5a suppression in diabetic limbal epithelial cells (LEC) enriched in LESC due to hypermethylation of WNT5A and up-regulation of the Wnt5a inhibiting miR-203a. In this study, we investigated the effects of DNA demethylation by zebularine, and nanopolymer based gene therapy using antisense oligonucleotide (AON) to inhibit miR-203a, on diabetic corneal epithelial wound healing and LESC expression. To this end, 5-mm wounds were created with 1-heptanol to remove the central corneal epithelium in diabetic organ-cultured corneas from postmortem human donor eyes that were pre-treated with either (A) DMSO control or DNA methylation inhibitor, zebularine (20 μM) for 48 hours or (B) biodegradable nanobioconjugates based on polymalic acid (PMLA) scaffold containing a control scrambled AON or AON to miR-203a for 4 days and subsequent wound healing was monitored over time. Healed corneas were embedded in OCT and sectioned (5 μm) for immunostaining to determine LESC and diabetic marker expression. We found that treatment of diabetic organ-cultured corneas with zebularine caused significant decrease in DNMT1 and increase in Wnt5a expression, accompanied by the acceleration of wound healing vs. DMSO controls. Furthermore, zebularine treatment resulted in an increase in the expression of putative LESC markers, keratins 15 and 17, as well as diabetic marker, nidogen-1, and activated the wound healing mediator p-Akt (S473). Likewise, treatment with the nanodrug PMLA-AON miR-203a also caused an increase in Wnt5a expression and putative LESC and diabetic markers and

stimulated wound healing vs. scrambled AON control. In conclusion, this study showed for the first time the dual epigenetic regulation of WNT5A in the diabetic cornea through DNA hypermethylation and miRNA action. Inhibition of DNA hypermethylation and miR-203a can normalize corneal epithelial wound healing and LESC expression through the upregulation of Wnt5a.

Funding Source: This work is supported by NIH/NEI grants R01 EY013431, R01 EY031377
Keywords: Corneal stem cells, DNA methylation, Diabetic wound healing

TOPIC: IMMUNE SYSTEM

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METABOLIC REGULATION OF BLOOD PROGENITOR HOMEOSTASIS AND HETEROGENEITY BY TCA CYCLE IN DEVELOPMENT AND IMMUNE RESPONSE IN DROSOPHILA LARVAE

Kumar, Ajay - *Regulation of Cell Fate, Institute for Stem Cell Science and Regenerative Medicine (inStem), Bangalore, India*

Abstract: Immunity as well as metabolism are quite old and extensively focused and sort after fields, but their inter-dependency, as Immune- metabolism, is being advocated very recently. This crosstalk infers how under immune compromised state, metabolic shift occurs and metabolites (a-KG, Succinate, Fumarate, etc.) takes on the tasks of proliferation, differentiation and activation of the concerned immune progenitors/cells. Recent work from our lab also highlighted GABA (released from brain) can elicit distinct immune cell population, under wasp infestation, which is nowhere to be seen in homeostasis. Through our work we would like to switch the conventional paradigm of looking at TCA cycle as just an “intermediary step in the glucose metabolism for energy production” to the “reservoir of cardinal immune metabolites”. Metabolites are well known to get exchanged between the cellular compartments, which brands them competent for being signalling molecules. And what could be the better place for studying metabolites than TCA cycle, which is source as well as sink for all the three macromolecules of life. Conventionally TCA cycle, as the name suggest, always considered to be a cycle, but is it actually a cycle or an important junction of various cycles, so as to facilitate the exchange as well as maintenance of constant concentration of each metabolite. If the later is true, then each of the metabolites generated here can be an independent signalling molecules and directs the heterogeneity of different system. Here with our thorough analysis of TCA cycle, we would like to shed some light onto the crosstalk between the intermediary metabolites, development of blood progenitors and the heterogeneity produced within.

Keywords: TCA Cycle, blood cells, heterogenity

TOPIC: NEURAL

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COMBINED EFFICACY OF LY294002 AND OTS964 IN SUPPRESSING SELF-RENEWAL OF TEMOZOLOMIDE-RESISTANT GLIOMA STEM CELL POPULATIONS

Sugimori, Michiya - *Integrative Neuroscience, University of Toyama/ Faculty of Medicine, Toyama, Japan*
Hayakawa, Yumiko - *Neurosurgery, University of Toyama, Japan*
Kuroda, Satoshi - *Neurosurgery, University of Toyama, Japan*

Abstract: Glioblastoma, a primary brain tumor, is resistant to chemotherapy and can develop into a fatal space-occupying lesion. Glioma stem cells (GSCs) are thought to be responsible for tumor growth, chemo-resistance, and recurrence. Clonal glioma sphere (GS) culture, in which GSCs are enriched and self-renew as GS clone populations, provides us with quantitative details regarding GS clone survivability and changes in growth during GS/GSC population self-renewal. Previously, we proposed a novel chemotherapeutic paradigm, temozolomide (TMZ) and OTS964 in combination (T&O), and showed that T&O efficiently eliminated self-renewing GS clones and significantly suppressed the regrowth of TMZ-sensitive GS clones. However, it remained unclear whether T&O would be effective in treatment of TMZ-resistant GSC populations. T&O did not suppress T98-GS clone growth during population self-renewal, suggesting that TMZ-like growth suppression is necessary for the long-term control of GSC population size. In this study, we tested the PI3K inhibitor LY294002, which is thought to suppress GSC self-renewal, alone and in combination with OTS964 (L&O) against T98G-GS populations. LY294002 alone suppressed T98G-GS clone growth for 2–3 weeks, while allowing the clones to survive. By contrast, L&O efficiently eliminated two-thirds of the T98G-GS clones and continuously suppressed T98G-GS clone regrowth for 2–3 times longer than LY294002 alone, suggesting that L&O represents an alternative to T&O. Moreover, T98G-GS clones pre-treated with L&O exhibited a half survival rate in the following generations, suggesting that L&O treatment perturb the GSC self-renewal capacity. Our findings indicate that this quantitatively validated combination paradigm could control growth of TMZ-resistant GSC populations through immediate and sustained shrinkage of GSC populations exhibiting power-law governed growth diversity.

Keywords: GLIOMA STEM CELL, POPULATION SELF-RENEWAL, TEMOZOLOMIDE-RESISTANT GLIOMA CELL

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

811

UNIQUE SUBPOPULATIONS OF SSEA-4+ / CD271+ CELLS DERIVED FROM WHARTON JELLY MESENCHYMAL STEM/STROMAL CELLS

Kaminska, Agnieszka - *Translational Platform of Regenerative Medicine, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*
Kominek, Agata - *Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
Piwocka, Katarzyna - *Laboratory of Cytometry, Nencki Institute*

of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
Sarnowska, Anna - *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

Abstract: Heterogenous mesenchymal stem/stromal cells (MSCs) population contains cells with different morphology, phenotype, differentiation abilities and even origin. From the therapeutic standpoint, isolation and application of specific subpopulations could be more effective than using the entire population. Here we isolated the specific cell subpopulations positive for SSEA-4 and CD271 derived from Wharton Jelly (WJ-MSC), a part of umbilical cord. Glycosphingoliphid SSEA-4 (specific stage embryonic antigen 4) appears during the early embryonic development, while CD271 (low-affinity nerve growth factor receptor) is proposed as a marker for cells derived from neural crest, a transitional structure formed during neurulation. The expression of both markers has been described previously but their characterization for MSC remains unrecognized. Proposed markers may indicate cells with unique properties and enhanced differentiational potential due to their distinct, early developmental origin. SSEA-4+ and CD271+ WJ-MSCs were separated with two methods: Fluorescence-activated Cell Sorting (FACS) with Cell sorter BD FACSAria II (BD) and Magnetic Activated Cell Sorting (MACS) with AutoMACS ProSeparator (Miltenyi Biotec). After separation, we analyzed obtained subpopulations: the number of cells positive for both markers, proliferation doubling time (PDT), clonogenic potential and persistence of SSEA-4/CD271 expression in subsequent passages. We observed that WJ-MSCs contain between 40-85% SSEA-4+ cells before the separation; the dispersion depends on the platelet lysate used in the culture medium. On the contrary, the population of CD271+ cells, although small (5%), does not depend on the lysate used. During cultivation time, SSEA-4 expression remains stable, while the number of cells expressing CD271 gradually decreases both in initial population and after cell sorting. Obtained subpopulation indicate 20%-30% clonogenic properties. WJ-MSC contains sufficiently numerous subpopulations of cells expressing markers appearing in early stages of development, what enables its isolation and cultivation. Further analysis of the obtained subpopulations may help to understand the nature of MSC.

Funding Source: National Science Centre grant no. NCN 2018/31/B/NZ4/03172; ESF, POWR.03.02.00-00-I028/17-00;
Keywords: Mesenchymal Stem/Stromal Cells, Specific Stage Embryonic Antigen 4, CD271

TOPIC: PLURIPOTENT STEM CELLS

813

SUPER-RESOLUTION IMAGING REVEALS TEMPORAL CHANGES IN CHROMATIN STRUCTURE AND GENE ACTIVITY IN SINGLE REPROGRAMMING CELLS

Martinez-Sarmiento, Jose A. - *Physiology, University of Pennsylvania, Philadelphia, PA, USA*

Abstract: During reprogramming, the predominantly compact chromatin landscape of somatic cells extensively reorganizes to acquire a pluripotent-like configuration; however, how chromatin is remodeled and its link with changes in gene expression dynamics are not well understood. This knowledge gap comes from the challenge of studying iPSC reprograming at the single cell level, since iPSC reprograming efficiency is low and it is difficult to identify the rare cells undergoing a correct reprogramming trajectory. Furthermore, key changes in chromatin spatial organization occur at nano-scale level, which cannot be visualized by conventional light microscopy. To overcome these limitations, we employed the highly efficient heterokaryon reprogramming system (fusion between somatic and pluripotent cells) to study, at the single-cell level, the gene reactivation dynamics and the spatial and epigenetic changes to chromatin at the onset of pluripotency conversion using super-resolution microscopy and RNA-FISH. Our results revealed that, within 6h and 48h following fusion of human fibroblasts with mouse ESCs, there is a staggered and dramatic decrease of the repressive histone modification marks H3K9me3 and H3K27me3 at the somatic nucleus. Surprisingly, active H3K4me3 and H3K9ac marks did not change during this time frame. These epigenetic changes preceded the spatial remodeling of chromatin, which underwent decondensation only at 48h after fusion (P = 0.0348). Notably, we detected nascent OCT4 RNA expression already at 6h and 12h after fusion, followed by mature RNA production at 24h and 48h in >70% of heterokaryons. In contrast, only 20% of heterokaryons exhibited NANOG reactivation across these time-points but at consistently low levels, suggesting different reactivation mechanisms for these genes. Altogether, our findings suggest that during reprogramming, cells undergo decommissioning of repressive histone modification marks followed by global chromatin decompaction. Moreover, the reactivation of some key pluripotency genes, such as OCT4, are uncoupled from and happen prior to the acquisition of a pluripotent-like open chromatin configuration. Future work will address what are the key differences in chromatin nano-structure at specific pluripotent gene regions during reprogramming using OligoSTORM.

Funding Source: CONACYT PhD Scholarship (Becas al Extranjero, Mexico) Linda Pechenik Montague Investigator Award (Melike Lakadamyali)
Keywords: Reprogramming, Super-resolution microscopy, Chromatin

815

KEY ROLE OF THE HIPPO/YAP PATHWAY IN HUMAN NAÏVE PLURIPOTENT STEM CELL MAINTENANCE AND TROPHECTODERM INDUCTION

Dattani, Anish - *Living Sytems Institute, Living Sytems Institute - University of Exeter, UK*
Huang, Tao - *Living Systems Institute, Living Systems Institute, University of Exeter, UK*
Smith, Austin - *Living Systems Institute, Living Systems Institute, University of Exeter, UK*
Guo, Ge - *Living Systems Institute, Living Systems Institute, University of Exeter, UK*

Abstract: Human naïve pluripotent stem cells (nPSCs) can readily differentiate into trophectoderm (TE). nPSCs are propagated in a combination of inhibitors of MEK/ERK (PD0325901), tankyrase (XAV939) and atypical protein kinase C (aPKC) (Gö6983) together with the cytokine LIF (collectively known as PXGL). Inhibition of MEK/ERK is critical to human nPSC self-renewal, yet paradoxically is also instrumental in TE differentiation. Therefore, and unlike mouse embryonic stem cells, robust self-renewal of human nPSCs requires suppression of TE induction. This is achieved by XAV939 and Gö6983. The functional effect of XAV939 is generally considered to be inactivation of canonical Wnt signalling through stabilisation of the β-catenin destruction complex. How-

ever, deletion of β-catenin does not alter the dependence of nPSCs on XAV939 nor diminish their ability to differentiate into TE. We found that in nPSCs tankyrase inhibition stabilises the HIPPO pathway component AMOTL2 and reduces nuclear localisation of YAP. Nuclear YAP is known to drive TE lineage segregation in the early embryo. We generated YAP knockout nPSCs and found that they fail to differentiate to TE. Furthermore, YAP deficient nPSCs can be stably propagated in MEK inhibitor alone without requirement for XAV939 or Gö6983. nPSCs can self-organise into a blastocyst-like structure ('blastoid') with high lineage fidelity to the human embryo. Using a robust platform for blastoid formation we show how YAP and the aPKC polarity complex regulate TE segregation and subsequent blastocoel formation. These findings explain the distinct requirements of human nPSCs for signalling inhibitors and illustrate the potential of the blastoid model for molecular genetic dissection of early human embryogenesis.

Keywords: HUMAN NAÏVE PLURIPOTENCY, TROPHECTODERM DEVELOPMENT, BLASTOID

TOPIC: MUSCULOSKELETAL

879

DISTINCT HYPOMETHYLATION PATTERNS OF OSTEOGENIC SPECIFIC PROMOTER GENES PROMOTE OSTEO-REGENERATIVE TENDENCY IN 3-DIMENSIONAL SPHEROIDS OF DENTAL PULP STEM CELLS

Raik, Shalini - *Biophysics, Post Graduate Institution of Medical Education and Research (PGIMER), Chandigarh, India*
Bhattacharyya, Shalmoli - *Biophysics, Post Graduate Institution of Medical Education and Research (PGIMER), Chandigarh, India*
Pal, Arnab - *Department of Biochemistry, Post Graduate Institution of Medical Education and Research, Chandigarh, India*
Rattan, Vidya - *Department of Oral Health Sciences, Post Graduate Institution of Medical Education and Research, Chandigarh, India*
Kumar, Navin - *Departmentment of Mechanical Engineering and Biomedical Engineering, Indian Institute of Technology Ropar, Rupnagar, Punjab, Ropar, Panjab, India*

Abstract: Regeneration and repair of bone defects is a cause of concern due to the limitations and poor bone healing outcomes of conventional bone graft-based treatment strategies. Growing number of evidences suggest that mesenchymal stem cells (MSCs) possess immense potential in repairing and restoration of damage tissue. However, the successful clinical outcome of cellular based therapy is dependent on the apt selection of stem cell in terms of type, source, origin, potency and method of their cultivation. In this context, the dental pulp stem cell represents a good alternative for normal bone regeneration due to their ecto-mesenchymal origin and expression of early markers of osteogenic lineage. Further, the physiological and biological aspects of stem cells are determined by the cellular microenvironment that vary between conventional and modern culture system i.e. 2-dimensional monolayer culture and 3- dimensional spheroid culture, eventually leading to distinct therapeutic efficacy. 3D spheroid tends to have better cell to cells and cell -matrix interactions which renders better environmental cues than the monolayer culture, ultimately promoting their regenerative abilities. Therefore, we studied the transcriptional and promoter methylation pattern of osteogenic gene during differentiation period in 3D spheroids and monolayer culture. We found enhanced expression of RUNX2, COL1A1, and OCN in 3D spheroid culture. The bisulfite sequencing results revealed difference in the methylation pattern between 2D and 3D culture derived DPSCs. OCN promoter showed significantly hypomethylated CpG site that could possibly lead to higher expression of OCN marker in DPSC spheroids during osteogenic differentiation. In addition, persistently low methylation level lead to the increased transcriptional pattern of osteogenic genes in DPSCs. Our in-vivo results in calvarial bone defect model in rat also confirmed superior bone formation in the defect region in animals implanted with spheroids compared to monolayer DPSCs. Overall, the results of this study indicate that the significant hypomethylation is associated with increased expression of osteogenic markers in spheroid culture that makes it a superior model for bone related regenerative applications.

Funding Source: None
Keywords: 3-dimensional spheroid culture, dental pulp stem cells, osteogenic differentiation

TOPIC: LIVER

881

NOVEL LIVER CANCER ORGANOIDS AS 3D IN-VITRO MODELS TO STUDY HEPATOCELLULAR CARCINOMA

El-Badri, Nagwa - *Biomedical Science, Zewail City of Science and Technology, October City, Egypt*
El-Derby, Azza - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Khedr, Menna - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*
Ghonem, Nehal - *Biomedical science, Zewail City for Science and Technology, Cairo, Egypt*

Abstract: Hepatocellular Carcinoma (HCC) is a leading cause of cancer-associated death worldwide so reliable experimental models should be developed to faithfully mimic the cancer microenvironment and shed light on the involved cellular cross-talk. Organoids are an excellent disease model to gain a deeper understanding of HCC pathogenesis and to develop novel therapeutics and biomarkers. Challenges for 3D model development for HCC include high cost, lengthy procedure, xeno contamination, and short life span due to lack of vascularization. In this work, we engineered HCC preclinical model to overcome some of these limitations. HCC organoids comprise liver parenchymal compartment, HUH-7, and non-parenchymal cells including human umbilical vein endothelial cells and human bone marrow-derived mesenchymal stromal cells. Organoids were cultured in different cell ratios with or without the non-parenchymal cells for 14 days in xeno-free natural biomatrix to provide the cells needed cues to grow and self-assembly. The resulted liver cancer organoids maintained viability for more than 29 days as tested by calcein stain and flow cytometry. Organoids that comprise vascular and stromal cells showed a significant level of HCC markers expression such as AFP, KRAS, c-Myc, c-Met, IGF2, and RHOA. There was a concomitant increase in invasion properties through augmented endothelial mesenchymal transition markers such as Snail, Vimentin, E-cadherin, and matrix metalloproteases accompanied with branched morphology acquired by organoids containing stromal compartment. The heterogeneous cancer organoid comprised cancer stem cells population as revealed by gene expression and immunocytochemistry data for cancer stem cells markers (CD24, CD44, EPCAM, and CD133) along with side pop-

ulation assay. In addition, The generated HUH7-organoid formed malignant sheets when cultured subcutaneously in nude mice for 15 days. These data suggest the validity of the generated model as a global, xeno-free, and cost-effective to study HCC, HCC-associated CSC, and anti-cancer drug testing.

Keywords: Organoid, Cancer organoid, Hepatocellular carcinoma

POSTER SESSION II: ODD

3:00 PM – 4:00 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

201

MAINTENANCE OF HOMEOSTATIC STROMAL PHENOTYPE AND ANTI-FIBROTIC PROPERTIES OF MSC USING SOFT-TISSUE ECM-MIMICKING ELASTIN-ALGINATE SCAFFOLDS

Xie, Yubing - *College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY, USA*
Ramesh, Pujhitha - *College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY, USA*
Moskwa, Nicholas - *Biological Sciences, University at Albany, State University of New York, Albany, NY, USA*
Zhang, Xulang - *College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY, USA*
Larsen, Melinda - *Biological Sciences, University at Albany, State University of New York, Albany, NY, USA*
Sharfstein, Susan - *College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY, USA*

Abstract: Mesenchymal stem/stromal cells (MSC) have anti-fibrotic and anti-inflammatory properties and have been used for many purposes in regenerative medicine. However, MSC tend to differentiate into disease-inducing myofibroblasts in a non-compliant environment, hampering MSC's regenerative potential. We hypothesize that a connective tissue extracellular matrix (ECM) mimicking, compliant microenvironment supports the homeostatic phenotype and anti-fibrotic properties of MSC. In previous work we engineered elastin-alginate cryoelectrospun scaffolds mimicking the topographic and viscoelastic properties of the connective tissue ECM of a healthy salivary gland tissue. Here, we used mouse embryonic day 16 (E16) mesenchyme cells as model MSC-like cells and cultured both healthy E16 mesenchyme cells and TGFβ1-induced fibrotic myofibroblasts in cryoelectrospun scaffolds. Cryoelectrospun scaffolds maintained homeostatic salivary stromal progenitor cell phenotype and suppressed the disease-associated myofibroblast phenotype. In particular, 3D co-culture of E16 mesenchyme in cryoelectrospun scaffolds (MSC-scaffold construct) with TGFβ1-treated myofibroblasts revealed that FGF2 further potentiated the anti-fibrotic potential of the MSC-scaffold construct. Altogether, we have demonstrated the potential of modulating the phenotype and maintaining the anti-fibrotic properties of MSC-like stromal cells with cryoelectrospun scaffolds, supporting future cell delivery of MSC for regenerative medicine applications.

Funding Source: This work is supported by National Institute of Health (NIH) National Institute of Dental & Craniofacial Research (NIDCR) under the grant number 1R01DE02795301 (M.L.).
Keywords: MSC, Elastin-alginate cryoelectrospun scaffold, anti-fibrosis

TOPIC: CARDIAC

203

INDUCED REPROGRAMMING OF ADULT MURINE CARDIOMYOCYTES TO PLURIPOTENCY AND PARTIALLY DE-DIFFERENTIATED STATES IN VIVO

de Lazaro, Irene - *Bioengineering, Harvard University, Cambridge, MA, USA*
Orejon-Sanchez, Tiara - *Bioengineering, Harvard University, Cambridge, MA, USA*
Tringides, Christina - *Bioengineering, Harvard University, Cambridge, MA, USA*
Mooney, David - *Bioengineering, Harvard University, Cambridge, MA, USA*

Abstract: Partial reprogramming of adult, somatic cells to a rejuvenated phenotype or to a transiently proliferative, progenitor-like state has been demonstrated in some mouse tissues by overexpressing Oct3/4, Klf4, Sox2 and cMyc (OKSM) transcription factors in situ. An approach like this applied to the mammalian heart could overcome the lack of endogenous cardiomyocyte turnover and regeneration that contributes to the poor resolution of heart disease, still the main cause of death worldwide. However, there is a negative correlation between a cell's differentiation status and its capacity to undergo reprogramming and in vivo reprogramming of adult mouse cardiomyocytes, highly differentiated and largely post-mitotic cells, has been elusive. Here, to solve this long-standing question, we combined cell type-specific Cre recombination and conditional, doxycycline-inducible, control of gene expression to generate cardiomyocyte-specific, inducible, reprogrammable mice with lineage tracing capabilities. We confirmed that eighteen days of doxycycline-induced OKSM expression established a gene expression program characteristic of the pluripotent state in the myocardium and triggered the generation of teratomas of confirmed cardiomyocyte origin in this model. However, shorter OKSM expression protocols induced different extents of cardiomyocyte de-differentiation (partial reprogramming) without tumorigenesis. Our findings confirm that the OKSM cocktail reprograms adult mouse cardiomyocytes to pluripotency and that the extent of reprogramming can be controlled by the duration of OKSM expression. Current work is investigating the contribution of partially reprogrammed cardiomyocytes to cardiac regeneration in a variety of heart disease models.

Keywords: in vivo reprogramming, cardiomyocytes, pluripotency

TOPIC: EPITHELIAL_LUNG

205

SMALL MOBILE STEM CELLS REGENERATING ALVEOLI IN EMPHYSEMA COPD RAT MODEL

Rahmo, Abdulkader - *Research and Development, SMSbiotech, San Marcos, CA, USA*

Abstract: Chronic obstructive pulmonary disease COPD is a progressive degenerative lung disease. The third or fourth leading cause of death worldwide, for which currently there is no effective cure or treatment. Small Mobile Stem (SMS) cells have demon-

strated strong and selective binding to key regenerative cells of the lung: alveolar epithelial type 2 progenitor cells and lung mesenchymal stem cells. The binding results in stimulation of proliferation and significant, meaningful, gene expression changes for both type of cells. SMS cell in vivo effect on the regeneration of lung alveoli have been examined using the Rat emphysema model induced using elastase enzyme. The instillation of SMS cells through the trachea resulted in significant regeneration of alveoli in the rat model. The histopathological parameter related to alveoli destruction, the mean linear intercept (MLI), was measured in paraffin embedded tissue cross-sections of inflated rat lungs. A shift from a MLI of 98 µm ($p < 0.001$), in elastase enzyme treated animals that have not been treated with SMS cells, to 65 µm ($p < 0.01$) in cell treated animals was observed. This was compared to MLI of normal non enzyme treated rats of 49 µm. This shift towards a significantly lower MLI value was achieved after only one week from SMS cell administration. Functional testing of the rat emphysema animal models using exercise further corroborated the histological measurements related to the alveoli destruction and regeneration, indicating significant improved performance in animals treated with SMS cells. SMS cells demonstrate, based on these data, the in vivo potential for significant lung tissue regeneration; coupled with the intrinsic resilience of these cells, further investigations could lead to a potential cell therapeutic treatment to emphysema in patients suffering from COPD.

Keywords: small mobile stem cells, COPD, stem cell stimulation

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

207

DATA REQUIREMENTS FOR HPSC LINES INTENDED FOR CLINICAL USE

Kurtz, Andreas - *IBMT, Fraunhofer Institute for Biomedical Engineering, Berlin, Germany*
Brandsten, Catharina - *Takara Bio Europe, Takara Bio Europe, Gothenburg, Sweden*
Bultjer, Nils - *IBMT, Fraunhofer IBMT, Berlin, Germany*
Freund, Christian - *Leiden University Medical Center, Leiden, Netherlands*
Hao, Jie - *Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
Hildebrandt, Martin - *Department of Internal Medicine III, Technical University Munich, Germany*
Kim, Jung Hyun - *Korean National Institutes of Health, Korean National Institutes of Health, Osong, Korea*
Ludwig, Tenneille - *WiCell Stem Cell Bank, Madison, WI, USA*
Mah, Nancy - *IBMT, Fraunhofer IBMT, Berlin, Germany*
Mueller, Sabine - *IBMT, Fraunhofer IBMT, Sulzbach, Germany*
Tennenbaum, Shelly - *The Hadassah Human Embryonic Stem Cell Research Centre, Hadassah University Medical Center, Jerusalem, Israel*
Stacey, Glyn - *ISCBI, ISCBI, Barley, UK*

Abstract: Human pluripotent stem cell (hPSC) lines are increasingly used as source material for clinical application. Currently, hPSCreg (https://hPSCreg.eu) lists 96 clinical trials using these cells. Data on the generation, manufacturing, quality control and used assays, genetic constitution and ethical and regulatory provenance of the hPSC lines used as source material is mostly not publicly available. In a provisional review of the likelihood of the existence and accessibility of data on hPSC - derivation and banking, a working group was formed, consisting of researchers involved in the development of hPSC lines intended for clinical use. The working group completed a questionnaire that asked about information and documentation on donor selection, cell derivation, banking procedures and testing. Responses from all seven responding centres indicated that they all had evidence for the existence of key documentation required at each of the key stages covered in the questionnaire. However, consistently, the biobanks could not make donor related information publicly available, although in some cases a summary could be made available, in one case some partial documentation could be made available and in two cases redacted consent documents were also publicly available. The biobanks varied in their ability to publicly release data and documentation on cell line isolation but again summaries were available or had already been published, or are shared upon request. At the levels of cell bank preparation and testing, the biobanks were more open to sharing such information publicly and some had published the results in detail although, it's independent inclusion in a separate database without linking to the source material may not be possible would require further discussion. Even access to test data would in general, require further work by some biobanks to obtain original documentation. The working group will continue to discuss ways to offer potential hPSC-product developers options for accessing available data.

Funding Source: European Commission Horizon2020 Project ID: 726320
Keywords: hPSC, data, clinical use

TOPIC: HEMATOPOIETIC SYSTEM

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MONITORING HYPOXIA USING IMMUNOFLUORESCENT IMAGING OF HIF-1α AND 8-OHDG AS CONTROLS FOR A WIDER HEMATOPOIETIC STEM CELL DETECTION ASSAY

Clarke, Sierra M. - *Gene Therapy, Research, and Clinical Bioanalytics, CSL Behring, Valencia, CA, USA*

Abstract: In the recent decades, cell and gene therapy has demonstrated a growing potential to treat diseases. As research continues to improve our understanding of the function of stem cells, hematopoietic stem cell (HSCs) culture conditions designed to replicate in-situ environments are being tested. In order to improve assays and reduce loss of stem cells prior to therapeutic use, CSL Behring tested the impact of hypoxia on HSC maintenance in vitro. HIF-1α and 8-OHdG marker expression were monitored, using immunostaining techniques to confirm the hypoxic conditions. HIF-1α, known to increase expression during cellular exposure to hypoxia, showed a 163% increase in fluorescence from normoxic conditions at the best dilution factor of 1:100. 8-OHdG, an expression of genomic DNA damage and cellular stress, decreased in hypoxic conditions. The results from this study will be used as control of future assay development design to identify HSCs from wider cell populations.

Funding Source: California Institute for Regenerative Medicine Bridges to Stem Cell Training Program California State University, Northridge CSL Behring
Keywords: hypoxia in hematopoietic stem cell culture, Hypoxia-inducible factor 1 alpha (HIF-1α), 8-hydroxy-2' -deoxyguanosine (8-OHdG)

TOPIC: LIVER

211

BIO-PRINTING OF PRIMARY HUMAN AND STEM CELL-DERIVED HEPATOCYTES AS A PLATFORM FOR LIVER DISEASE DIRECTED CELL THERAPY

Campbell, Stephanie A. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Ogawa, Mina - *McEwen Centre for Regenerative Medicine, University Health Network, Toronto, ON, Canada*
Tong, Haley H.L. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Agarwal, Rishima - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Kim, Taehyun - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Al-arabi, Lila - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Dickman, Christopher T.D. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Beyer, Simon T. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Mohamed, Tamer - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Wadsworth, Sam J. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*
Ogawa, Shinichiro - *McEwen Centre for Regenerative Medicine, University Health Network, Toronto, ON, Canada*
Witek, Rafal P. - *Aspect Biosystems Ltd., Vancouver, BC, Canada*

Abstract: Liver disease progression to liver failure is a global health concern that primarily affects hepatocytes, the main parenchymal liver cells. The major challenges for developing a liver cell therapy are the insufficient availability of hepatocytes and the lack of a robust technology to deliver them to patients. Recent advancements in stem cell differentiation towards hepatocyte-like cells have shown promise for production of metabolically functional cells that are similar to primary human hepatocytes. In addition, 3D bioprinting provides a consistent manufacturing process for generating implantable grafts that contain a defined number of hepatocytes encapsulated in unique biomaterials for therapeutic applications. In this study, we tested the hypothesis that Aspect's microfluidic 3D bioprinting technology allows for consistent printing of human embryonic stem cell (hESC)-derived hepatocytes and promotes their maturation when benchmarked against primary human hepatocytes and the HepG2 hepatic cell line. Using an established, patent-pending protocol, hESCs were differentiated to hepatocyte-like cells (hepatoblasts, zone I and III hepatocytes). Primary human hepatocytes and HepG2 cells were obtained from commercial vendors. All hepatic cell types were aggregated in 3D, combined with alginate-based biomaterial, and printed in core-shell structures using the Aspect RX1 bioprinter. All printed cells displayed high levels of albumin secretion and viability. As expected, primary hepatocytes displayed decreased levels of AFP secretion whereas HepG2 secreted high levels of AFP. Interestingly, 3D printed hepatocyte-like cells displayed increased levels of maturation relative to unprinted aggregates and HepG2 cells. This was evident by a reduction in AFP and an increase in albumin. Additional experiments are currently underway to determine in vitro and in vivo function of the 3D printed cells. Although hESC-derived hepatocytes do not completely replicate the high-level functionality of primary human hepatocytes, our data shows that by aggregation and printing in 3D, additional maturation ques are provided to increase their function and maturity. Further, high level viability and ease of printing makes hESC-derived hepatocytes highly desirable for use in solid organ cell therapy.

Keywords: 3D Bioprinting, Hepatocytes, Cell Therapy

TOPIC: NEURAL

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TRANSLATION OF A FETAL-DERIVED NEURAL PROGENITOR CELL PRODUCT FOR MULTIPLE NEURODEGENERATIVE DISEASES

Avalos, Pablo - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai, Los Angeles, CA, USA*
Lewis, Richard - *Neurology, Cedars-Sinai, Los Angeles, CA, USA*
Svendsen, Clive - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai, Los Angeles, CA, USA*
Wang, Shaomei - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai, Los Angeles, CA, USA*

Abstract: Neurodegenerative diseases collectively affected approximately 6.0 million individuals and were responsible for 272,644 deaths and 3,011,484 disability adjusted life years in the US in 2016. The economic burden to the U.S. of neurodegenerative diseases exceeds $655 billion including costs from lost productivity and uncompensated caregiving hours. Treatments currently available can relieve some of the symptomatology, however, there are no known cures. Additionally, there aren't effective treatments for diseases like amyotrophic lateral sclerosis (ALS). We have developed a cell therapy with the potential to treat multiple neurodegenerative diseases such as ALS, Parkinson's disease, and retinitis pigmentosa. Human fetal cortical-derived neural progenitor cells were expanded under cGMP (CNS10-NPC) and transduced with lentivirus encoding glial cell line-derived neurotrophic factor GDNF (CNS10-NPC-GDNF). Extensive preclinical studies have transplanted these cells into animal models of ALS (spinal cord and motor cortex transplantation), Parkinson's Disease and retinitis pigmentosa, where the cells have demonstrated efficacy. Under an IND, CNS10-NPC-GDNF were unilaterally transplanted into the lumbar spinal cord of 18 ALS subjects in a Phase 1/2a dose escalation study using a new minimally invasive surgical device. Quantifying muscle strength over time demonstrated that loss of strength was significantly delayed, in the treated leg, in the low dose cohort at the 12-month time point. This study reached the primary endpoint of safety and is the first combined gene and cell therapy approach for ALS to show both cell survival and growth factor release for up to 42 months. Currently, we are performing two additional Phase 1/2a dose escalation trials: one transplanting CNS10-NPC-GDNF unilaterally into the hand knob area of the motor cortex of patients with ALS and one transplanting CNS10-NPC into the subretinal space of patients with retinitis pigmentosa. At the same time, we are completing the IND-enabling studies for the use of CNS10-NPC-GDNF in Parkinson's disease and retinitis pigmentosa.

Funding Source: California Institute of Regenerative Medicine, U.S Department of Defense
Keywords: Neurodegenerative Disease, transplantation, cell therapy,

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A SMART HYDROGEL TO IMPROVE ACTIVATION OF A SUICIDE GENE TARGETED AT ENHANCING SAFETY OF HUMAN STEM CELL GRAFTS FOR BRAIN REPAIR

Law, Kevin - *The Florey Institute of Neuroscience and Mental Health, University of Melbourne, West Melbourne, Australia*
Mahmoudi, Negar - *School of Engineering, Australian National University, Canberra, Australia*
Nagy, Andras - *Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*
Thompson, Lachlan - *The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Australia*
Nisbet, David - *Graeme Clark Institute, The University of Melbourne, Australia*
Parish, Clare - *The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Australia*

Abstract: Advancements in human pluripotent stem cells (hPSCs) differentiation protocols to generate ventral midbrain (VM) dopamine progenitors have progressed the field of stem cell transplantation in Parkinson's disease. Despite this, resultant grafts in the brain yield low proportions of dopamine neurons and have a tumorigenic risk associated with the potential presence of incompletely patterned, proliferative cells within grafts. We recently demonstrated that donor cells carrying a suicide gene (thymidine kinase, TK) can improve the safety and standardisation of neural grafts by timely ablation of graft-derived proliferative cells undergoing S-phase. A key challenge however was coordinating activation of the suicide gene by the prodrug, ganciclovir (GCV), given the short S-phase of neural progenitors and short half-life of GCV. To address this, we engineered a series of smart hydrogels targeted at sustaining GCV delivery at the graft site in the brain. Hydrogels were optimised for stiffness and subsequently GCV delivery (comparing burst, slow sustained, and a combination of burst and sustained GCV release). All three hydrogels encapsulating GCV were capable of ablating hPSCs (carrying the TK suicide gene) as well as VM progenitors in vitro, demonstrating retained drug functionality following gel incorporation and release. Implantation of the GCV-loaded hydrogels into the mouse brain similarly ablated proliferating hPSCs in undifferentiated grafts. We are currently investigating the capability of these optimised GCV-functionalised gels to ablate incompletely patterned, proliferative cells within VM progenitor grafts, whilst simultaneously protecting the post-mitotic dopamine neurons necessary for repair in the Parkinsonian brain. These findings demonstrate the capacity of a functionalised biomaterial to provide enhanced drug delivery and activation of a suicide gene-based system, targeted at improving the safety of hPSC-derived neural grafting for brain repair.

Keywords: Neural transplantation, Biomaterials, Suicide gene

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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EVALUATING POSTNATAL REGENERATIVE CELLULAR THERAPY FOR SPINA BIFIDA IN A NATURALLY OCCURRING CANINE TRANSLATIONAL MODEL

Clark, Kaitlin C. - *Surgery, University of California, Davis, Sacramento, CA, USA*
Clark, Kaitlin - *Surgery, University of California, Davis, Sacramento, CA, USA*
Reed, Camille - *Surgery, University of California, Davis, Sacramento, CA, USA*
Garcia-Nolen, Tanya - *Surgical & Radiological Sciences, University of California, Davis, CA, USA*
Kreutzberg, Nicole - *Surgery, University of California, Davis, Sacramento, CA, USA*
Amador, Ashley - *Surgery, University of California, Davis, Sacramento, CA, USA*
Kumar, Priyadarsini - *Surgery, University of California, Davis, Sacramento, CA, USA*
Even, Lisa - *Veterinary Institute for Regenerative Cures, University of California, Davis, CA, USA*
Walker, Naomi - *Pathology, Microbiology and Immunology, University of California, Davis, CA, USA*
Agape-Toupadakis Skouritakis, Chrisoula - *Surgical & Radiological Sciences, University of California, Davis, CA, USA*
Vanover, Melissa - *Surgery, University of California, Davis, Sacramento, CA, USA*
Long, Connor - *Surgery, University of California, Davis, Sacramento, CA, USA*
Lankford, Lee - *Surgery, University of California, Davis, Sacramento, CA, USA*
Martins Amorim, Rogério - *Pathology, Microbiology, Immunology, University of California, Davis, CA, USA*
Lee, Gyehyun - *Surgery, University of California, Davis, Sacramento, CA, USA*
Borjesson, Dori - *Pathology, Microbiology, Immunology, University of California, Davis, CA, USA*
Stover, Sue - *Surgical and Radiological Sciences, University of California, Davis, CA, USA*
Farmer, Diana - *Surgery, University of California, Davis, Sacramento, CA, USA*
Sturges, Beverly - *Surgical and Radiological Sciences, University of California, Davis, CA, USA*
Wang, Aijun - *Surgery, University of California, Davis, Sacramento, CA, USA*

Abstract: The canine is increasingly recognized as a valuable pre-clinical large animal model for many human diseases. Canine spina bifida (SB) clinically presents very similarly to human SB, and English bulldogs in particular have a high incidence of naturally occurring SB. Placental mesenchymal stem cells (PMSCs) are being investigated as an adjunct to prenatal repair of SB; however, similar treatments have not been explored for postnatal repair. English bulldogs could serve as the first postnatal animal model of SB. The goal of this study is to evaluate canine PMSCs (cPMSCs) to test their efficacy as a postnatal therapy in a naturally occurring large animal disease model. To date, we have enrolled 2 normal controls and twelve 10-week-old English bulldogs with SB defects confirmed by neurological evaluation and magnetic resonance imaging (MRI). Each SB dog underwent a multi-segment lami-

nectomy and 7/12 dog's treatment was coupled with transplant of allogeneic cPMSCs embedded in hydrogel and extracellular matrix scaffold. One-year follow-up has been completed on ten study animals and the remaining two animals are currently being evaluated. The first two dogs enrolled were initially ambulatory with notable abnormal gaits and incontinence. MRI revealed L7-S1 defects of varying severity. One MRI also showed a large syringohydromyelnia involving the lumbar spinal cord. Electrophysiologic testing revealed low-normal conduction velocity for both motor and sensory hindlimb nerves. All dogs recovered from posterior laminectomy and cPMSC implantation uneventfully. At 8 weeks post-treatment all dogs showed improved ambulatory gaits, with final gait analysis pending. MRIs performed at 8 weeks showed only syrinx recurrence. No significant adverse events occurred in any dog by 12 months. The remaining animals' evaluations are currently being collected and/or analyzed. Postnatal treatment of a naturally occurring canine model of SB with allogeneic cPMSCs is clinically feasible and appears safe. Further studies are currently being performed to evaluate efficacy. The findings from this study suggest that naturally occurring canine SB is a valuable translational model to evaluate PMSC postnatal therapy and will provide critical insights for human clinical studies.

Funding Source: UC Davis CCAH and VIRC, NIH (5R01NS100761-02), Shriners Hospitals for Children (85108-NCA-19), Lodric Maddox Fellowship, National Center for Advancing Translational Sciences (UL1 TR001860) and the Willis&Ethel Clark Foundation.
Keywords: Spina Bifida, Mesenchymal Stem/Stromal Cell, Placenta

TOPIC: PLURIPOTENT STEM CELLS

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DEVELOPMENT OF A VASCULARISED, COMPLEX HUMAN 3D IN VITRO SKIN BY COMBINING IPSC SKIN ORGANOIDS AND A MESODERMAL PROGENITOR CELL-DERIVED VASCULAR NETWORK

Reigl, Amelie - *Tissue Engineering and Regenerative Medicine, Fraunhofer ISC, Würzburg, Germany*
Wörsdörfer, Philipp - *University Würzburg, Anatomy Instutute, Würzburg, Germany*
Zdzieblo, Daniela - *Fraunhofer ISC, Wuerzburg, Germany*
Metzger, Marco - *Tissue Engineering and Regenerative Medicine, Fraunhofer ISC, Würzburg, Germany*
Groeber-Becker, Florian - *Fraunhofer ISC, Wuerzburg, Germany*
Groneberg, Dieter - *Tissue Engineering and Regenerative Medicine, Fraunhofer ISC, Würzburg, Germany*

Abstract: Current in vitro skin model consists of the two skin cell types, i.e., primary epidermal keratinocytes and dermal fibroblasts isolated from human foreskin. The fibroblasts are usually combined with a collagen matrix to mimic the dermal layer. Subsequently keratinocytes are seeded on top to form a functional epidermis. These three-dimensional full-thickness skin equivalents (FTSE), cultured at the air-liquid interphase, are well established for risk assessment and efficacy testing. However, a mayor pitfall of these models is their low complexity, as not all cell types of human skin are included. Therefore, we want to use complex human skin organoids derived from induced pluripotent stem cells (iPSCs), which offer unique opportunities for mechanistic studies of skin development and various skin diseases that complement animal studies. To improve our in vitro skin model, we used skin organoids that mimic complex skin tissue via cell-cell interaction among different cells by releasing chemical signals to trigger the complex maturation of the organ. We already identified different cell types after the successful differentiation. We could find keratinocytes and hairpeg formation (CK5, CK17), dermal cells (vimentin) and adipocytes (nile red). Moreover, we used iPSC-derived vascular organoids and introduced vasculature into the in vitro skin models. We characterized the cell types with different makers for endothelial cells (CD31), smooth muscle cells (SMMHC), pericytes (NO-GC) and fibroblasts (desmin). The combination of both established organoid models as in vitro 3D reconstructed vascularized skin will shed light on the process of angiogenesis, investigate hair development and will help test drugs to treat skin defects and improve skin regeneration.

Funding Source: Institutes Fraunhofer Institute for Biomedical Engineering IBMT, the Fraunhofer Institute for Silicate Research ISC, the Fraunhofer Project Center for Stem Cell Process Engineering
Keywords: organoids, skin, vascularization

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A DECADE IN THE MAKING: 10-YEAR STABILITY ASSESSMENT OF CRYOPRESERVED, SINGLE-CELL DERIVED MULTIPLEXED ENGINEERED IPSC BANKS

Fong, Lauren - *Cellular Reprogramming and Engineering, Fate Therapeutics, San Diego, CA, USA*
Brookhouser, Nicholas - *Cellular Reprogramming and Engineering, Fate Therapeutics, San Diego, CA, USA*
Lai, Yi-Shin - *Cellular Reprogramming and Engineering, Fate Therapeutics, San Diego, CA, USA*
Robinson, Megan - *Cellular Reprogramming and Engineering, Fate Therapeutics, San Diego, CA, USA*
Huffman, Janel - *Molecular Engineering, Fate Therapeutics, San Diego, CA, USA*
Lee, Tom - *Molecular Engineering, Fate Therapeutics, San Diego, CA, USA*
Abujarour, Ramzey - *Cellular Reprogramming and Engineering, Fate Therapeutics, San Diego, CA, USA*
Valamehr, Bahram - *Research & Development, Fate Therapeutics, San Diego, CA, USA*

Abstract: Induced pluripotent stem cells (iPSCs) are an ideal renewable source of starting material for the mass production of uniform and consistent multiplexed-engineered cellular therapies. Unlike primary natural killer (NK) and T cells that have limited proliferative capacity and clonogenicity, iPSCs are readily amenable to complex manipulations such as genetic engineering, cloning, and large-scale expansion without losing their pluripotent potential or self-renewal capacity. Over the past decade, we have developed a robust proprietary iPSC platform, where cellular reprogramming, maintenance of pluripotency in the naïve state, and single-cell culture in a feeder-free environment is enabled by stage-specific, small molecule combinations to block differentiation, enhance survival, and support self-renewal of iPSCs. We have applied our iPSC product platform to generate clonal master iPSC banks and have used these banks for cGMP manufacture of multiplexed-engineered, off-the-shelf NK and T-cell product candidates now in clinical studies. Establishing a bank stability program to ensure the long-term availability and viability of clonal master iPSC banks for drug product manufacturing is critical to ensure safety, efficacy, and manufacturing demands throughout

a product's life cycle. To this end, we have performed long-term stability studies of cryopreserved iPSC banks. Tested iPSC banks were generated using different starting cell types, different reprogramming methods, and different methods of engineering. Regardless of the starting material or the methods used, iPSC banks generated using our naïve reprogramming platform maintained stability for at least the period tested (>10 years). Stability was assessed by examining critical quality attributes including viability, purity, potency, and phenotypic and genomic stability. The stability of transgene expression in iPSCs following engineering and long-term cryopreservation was examined and iPSCs were found to maintain stable transgene expression over extended banking intervals. Collectively, our study supports the great utility of iPSC lines created using our proprietary iPSC platform and maintained under our banking paradigm as a renewable and long-lasting starting material for the generation off-the-shelf cell therapies.

Keywords: iPSC, stem cells, stability

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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LOW RESULT REPORTING RATES OF THE CLINICAL TRIAL OF REGENERATIVE MEDICINE REGISTERED ON CLINICALTRIALS.GOV: AN ANALYSIS USING ADISINSIGHT DATABASE

Negoro, Takaharu - *Center for Reverse Translational Research, Osaka Habikino Medical Center, Habikino, Japan*
Okura, Hanayuki - *Institute of Innovative Medical Technology, Osaka, Japan*
Hayashi, Shigekazu - *Center for Reverse Translational Research, Osaka Habikino Medical Center, Habikino, Japan*
Arai, Tsutomu - *Center for Reverse Translational Research, Osaka Habikino Medical Center, Habikino, Japan*
Matsuyama, Akifumi - *Institute of Innovative Medical Technology, Osaka, Japan*

Abstract: Sharing the protocols and results of clinical trials with full transparency is an ethical obligation of those involved in clinical research. In our previous trend analysis in regenerative medicine associated strokes, the result reporting rate of trials on gene and/or cell therapy was suspected to be low. In this analysis, we used AdisInsight (Springer Nature) to expand the scope of trials registered in ClinicalTrials.gov to all disease areas. AdisInsight is a commercial database that supports drug discovery that provides the information being developed by companies and their collaborating academic institutions around the world. We extracted trials actually completed from 2011 up to 2020 on ClinicalTrials.gov. Furthermore, since the drugs listed in AdisInsight are categorized by drug category, we conducted a comparative analysis of the result reporting rate of gene therapy and cell therapy on the ClinicalTrials.gov, categorizing treatment modalities, which are biologics (as a control), gene therapy, and cell therapy studies. As a result, the result reporting rate for biologics, gene therapy, and cell therapy was 68%, 49%, and 35%, respectively, with each difference being significant ($p < 0.05$). Our initial suspicion that the result reporting rate might be low in the regenerative medicine trials registered in ClinicalTrials.gov was confirmed. We consider that more detailed research to identify the factor of the poor reporting rate and improve the issue will be needed.

Funding Source: This work was supported by the Highway Program for Realization of Regenerative Medicine of The Japan Agency for Medical Research and Development (AMED) under Grant Number JP18bm0504009.
Keywords: Result reporting of trials on RM may be low, RM trials registered in ClinicalTrials.gov, comparative analysis of CT, GT and biologics

TOPIC: IMMUNE SYSTEM

819

CELL-BASED PASSIVE IMMUNIZATION FOR LONG-TERM PROTECTION AGAINST COVID-19

Sawula, Evan - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Miersch, Shane - *The Donnelly Centre, University of Toronto, ON, Canada*
Jong, Eric - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Li, Chengjin - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Chou, Grace - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Saberianfar, Reza - *The Donnelly Centre, University of Toronto, ON, Canada*
Tang, Jean Kit - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Harding, Jeffrey - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Lye, Kathryn - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*
Sidhu, Sachdev - *The Donnelly Centre, University of Toronto, ON, Canada*
Nagy, Andras - *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON, Canada*

Abstract: Immunologically impaired individuals respond poorly to COVID-19 vaccines, which underscores the need for alternative strategies to protect these vulnerable populations from SARS-CoV-2 infection. While passively administered antibodies can confer pre-exposure prophylaxis, their short lifespan in patients limits the duration of immune protection. Combining cell engineering with monoclonal antibody technologies offers a unique opportunity to provide lasting passive immunity with long-lived cell implants. Here, we show that pluripotent stem cells engineered for safety and allogeneic acceptance can secrete potent SARS-CoV-2 neutralizing biologics (nBios). These include multivalent nBio formats with enhanced neutralization breadth and potency to combat SARS-CoV-2 variants. Clonal derivatives of the engineered stem cells act as in vivo delivery vehicles by sustainably supplying protective levels of nBios to isogenic and allogeneic mice as subcutaneous cell implants. As such, we anticipate that mice harboring implants will be protected from SARS-CoV-2 in upcoming in vivo infection studies. Altogether, these findings demonstrate the promise and potential of using "off-the-shelf" cell products that secrete neutralizing antibodies to long-term protect against current viral threats, including SARS-CoV-2, and other viruses that may emerge in the future.

Keywords: Cell therapy, Passive immunization, COVID-19

TOPIC: NEURAL

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VASCULARIZATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED OTIC NEURONAL SPHEROIDS USING A BIOMIMETIC SCAFFOLD

Norton, Benjamin M. - *Otolaryngology - Head & Neck Surgery, Northwestern University, Oak Park, IL, USA*
Matsuoka, Akihiro - *Otolaryngology - Head & Neck Surgery, Northwestern University, Chicago, IL, USA*

Abstract: Transplantation of three-dimensional (3D) human pluripotent stem cell (hPSC)-derived otic neuronal progenitor (ONP) organoids is a promising treatment strategy for sensorineural hearing loss. We have previously demonstrated that assembly of hPSC-derived ONPs into 3D spheroids confers improved survival after transplantation in the harsh cochlear microenvironment. However, this arrangement does not permit adequate oxygen and nutrient supply to the spheroid core, resulting in core hypoxia and necrosis and thereby limiting the therapeutic potential of this method. To overcome this challenge, we investigated a combinatorial approach in which a vascular network generated from human induced pluripotent stem cell (iPSC)-derived endothelial cells (ECs) was integrated with a novel 3D biomimetic hydrogel organoid scaffold (Prellis Biologics, Hayward, CA, USA) to facilitate oxygen and nutrient delivery and support spheroid survival. Spheroids were generated by sequentially seeding hydrogel scaffolds with iPSC-derived ONPs and Matrigel-embedded ECs. To promote angiogenesis directed toward the spheroid core, POlyhedrin Delivery System (PODS®; Cell Guidance Systems, Cambridge, UK) co-crystals secreting vascular endothelial growth factor (VEGF) were encapsulated within the spheroid. Results indicate that ECs self-organized into vessel-like structures and migrated into the spheroid core. Furthermore, vascularization was associated with improved survival in vitro. Together, these results implicate this method of prevascularization as a candidate strategy for improving long-term survival in vivo following transplantation into the cochlea. Future studies will further assess the perfusability of microvasculature and post-transplantation survival in murine cochleae.

Keywords: vascularization, tissue engineering, inner ear regeneration

TOPIC: PLURIPOTENT STEM CELLS

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ADOPTIVE TRANSFER OF IPSC-MACROPHAGES RESCUES MICE FROM GRAM-POSITIVE AND GRAM-NEGATIVE PNEUMONIA

Rafiei Hashtchin, Anna - *Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany*
Ackermann, Mania - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*
Mannstein, Felix - *Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover Medical School, Hannover, Germany*
Kempf, Henning - *Department of Stem Cell Biology, Novo Nordisk, Måløv, Denmark*
Hetzel, Miriam - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*
Fehlhaber, Beate - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*
Bach, Luisa - *Institute of Hematology/Oncology, Universitätsklinikum Bonn, Bonn, Germany*
Meyer, Greta - *Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany*
Schambach, Axel - *Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany*
Zweigerdt, Robert - *Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover Medical School, Hannover, Germany*
Hansen, Gesine - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*
Viemann, Dorothee - *Translational Pediatrics, Department of Pediatrics, University Hospital Würzburg, Würzburg, Germany*
Munder, Antje - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*
Lachmann, Nico - *Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany*

Abstract: According to the World Health Organization (WHO), lower respiratory tract infections (LRTIs) remain among the world's most deadly communicable disease, with more than 2 million deaths worldwide in 2020. Especially, LRTI caused by multi-drug-resistant bacteria represent a serious problem, which highlights the unmet need for novel and alternative treatment options. Host-directed therapies either to foster the function of endogenous immune cells or to support the endogenous immunity by direct transfer of immune cells represent new and promising therapeutic avenues. Considering the key role of alveolar macrophages, a pulmonary transfer of macrophages could be considered a promising strategy and induced pluripotent stem cells (iPSC) could serve as a standardized cell source for the generation of allogeneic off-the-shelf macrophages (iMonoMac). Using state-of-the-art manufacturing platforms, we could previously demonstrate the scalable and continuous generation of iMonoMac. The robust differentiation procedure resulted in the generation of 1-4x107 highly pure CD45+CD11b+CD14+CD163+ iMonoMac per week, which shared a similar morphology and transcriptomic profile with monocyte-derived macrophages (MDM). Recent in vitro studies revealed superior anti-bacterial activity of iMonoMac against Pseudomonas aeruginosa and Staphylococcus aureus compared to MDM. Whole transcriptome analysis of iMonoMac indicated a more profound upregulation of inflammatory genes early after infection and faster normalization 24 hours post-infection. This observation was in line with effective clearance of lab strains and clinical isolates of P. aeruginosa and S. aureus (incl. MRSA) within 24 hours post-infection. Importantly, pulmonary transfer of iMonoMac into P. aeruginosa or S. aureus pulmonary infected immunodeficient mice rescued mice from clinical symptomatic respiratory infections as shown by significantly reduced disease scores, lung bacterial load, normalized body temperature and lung function. Of note, the anti-bacterial properties of iMonoMac could even be further enhanced by preloading of the cells with the antibiotics Gentamicin and Levofloxacin. In summary, we provide novel concepts for macrophages-based immunotherapeutic approaches targeting pulmonary infections.

Keywords: Macrophages, Induced pluripotent stem cells, Pulmonary infection

POSTER SESSION II: ODD

3:00 PM – 4:00 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

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TRI-CELLULAR CARDIAC MICROTISSUES FROM HIPSCS MODEL POST-NATAL AND MULTICELLULAR CARDIAC ARRHYTHMIC DISEASE

Bellin, Milena - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Meraviglia, Viviana - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Campostrini, Giulia - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Ward-van Oostwaard, Dorien - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Mummery, Christine - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Human iPSC-derived cardiomyocytes (hiPSC-CMs) provide many advantages over previous in vitro systems for cardiac disease modelling, however they are immature and cannot capture non-CM contribution to disease. Here, we addressed these issues using a three-dimensional (3D) cardiac microtissue (MT) system composed by hiPSC-derived CMs, cardiac fibroblasts (CFs) and endothelial cells. This tri-cellular MTs were previously shown to promote structural, functional and gene expression maturation of hiPSC-CMs. We first built MTs using hiPSC-CMs carrying a mutation located in the adult splicing isoform of the cardiac sodium channel SCN5A. The SCN5A gene has two mutually exclusive exons, 6A expressed in fetal and 6B in adult CMs. We showed that exon 6B was only expressed in the 3D MTs and hiPSC-CMs isolated from the MTs revealed alterations in the sodium current, as analysed by patch clamp . We also demonstrated that the isoform switch was driven by the RNA-binding protein MBNL1. Since all MT components are differentiated from hiPSCs, we exploited this system to study arrhythmogenic cardiomyopathy (ACM), a complex multicellular genetic disorder, often presenting with cardiac arrhythmia and sudden death. We generated MTs by sequentially replacing either hiPSC-CMs or hiPSC-CFs or both, with cells carrying a mutation in PKP2 gene. The presence of one mutant cell type was sufficient to alter gene expression, evaluated by RNA-sequencing , in the pathways of protein membrane localization, sarcomere organization and extracellular matrix, and it affected the ability of MTs to respond to high stimulation frequencies. Interestingly, only the presence of both mutant cell types caused arrhythmia at high frequency. In conclusion, our data demonstrate the utility of the tri-cellular MT system for modelling post-natal cardiac disease, promoting maturation required to study cardiac arrhythmias due to mutations in adult gene isoforms, and for dissecting cell-type contributions of complex multicellular cardiac diseases.

Funding Source: European Union's Horizon 2020 research and innovation programme under European Research Council (ga No. 101001746) and ERA-NET Co-fund action No. 680969 (ERA-CVD JTC2016); The Novo Nordisk Foundation grants (NNG21CC0073729)
Keywords: cardiac microtissues, SCN5A, arrhythmogenic cardiomyopathy

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SINGLE CELL MULTI-OMICS REVEAL NOVEL CARDIAC SUBPOPULATION CRITICAL TO VALVE MORPHOGENESIS

Merriman, Alexander - *Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Costa, Mauro - *Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Ye, Chun - *Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA*
Srivastava, Deepak - *Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Congenital and acquired valvular heart disease are major sources of morbidity and mortality with an anticipated increase in prevalence secondary to an aging population and increase in survivorship for patients with congenital disease. Further developmental studies are required to advance novel therapeutic development including living tissue engineered heart valves and pharmacologic interventions. In this study, we have interrogated the later stages of valvulogenesis, following formation of the endocardial cushions, to understand the molecular mechanisms of valve formation and how these mechanisms are disrupted in the context of disease. Leveraging a combination of single cell RNA/Chromatin Accessibility sequencing in the developing mouse heart, we identified a novel, rare cell population in the developing valve with a unique transcriptional profile comprised of highly specific developmental signaling pathway genes. These cells are first detectable after valve primordia formation at embryonic day (E) 12.5 and are spatially localized at the leading edge of the developing leaflets. Ablation of this rare subpopulation during development results in highly dysplastic valves, characterized by hyperplastic, redundant, immature leaflets associated with valvular stenosis and regurgitation. These dysplastic features are consistent with the features of several congenital valvulopathies including Ebstein's Anomaly, and pulmonary or aortic valve stenosis. Single cell RNA sequencing analysis of a human fetal heart with hypoplastic left heart syndrome and critical aortic stenosis demonstrated a depletion of this cell population in the diseased aortic valve, suggesting these cells may be required for normal human valvular development as well. This study establishes the existence of a novel, rare subpopulation of cardiac cells that are critical to valve development and may contribute to the pathogenesis of congenital valvulopathies.

Funding Source: NIH/NHLBI F30HL158170
Keywords: Valvulogenesis, Cardiogenesis, Congenital Heart Disease

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MODELING MULTI-LINEAGE HEART FIELD DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELLS (HPSC)

Yang, Donghe - *Medical Biophysics, University of Toronto, ON, Canada*
Gomez, Juliana - *Biomedical Engineering, University of Toronto, ON, Canada*

Funakoshi, Shunsuke - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*
Tran, Thinh - *Molecular Genetics, University of Toronto, ON, Canada*
Fernandes, Ian - *Medical Biophysics, University of Toronto, ON, Canada*
Bader, Gary - *Molecular Genetics, University of Toronto, ON, Canada*
Laflamme, Michael - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*
Keller, Gordon - *McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada*

Abstract: The cardiomyocyte subtypes in the mammalian heart arise from distinct lineages known as the first heart field (FHF), the anterior second heart field (aSHF), and the posterior second heart field (pSHF) which are specified during gastrulation. To access different subtypes of human cardiomyocytes, we modeled human heart field development from hPSCs using single-cell RNA sequencing to delineate lineage specification and progression. Integration of human fetal, mouse, and hPSC-derived mesoderm transcriptomes enabled the identification of distinct human FHF, aSHF, and pSHF mesoderm subpopulations that gave rise to respective progenitors which in turn generated specific cardiomyocyte derivatives including left ventricular, right ventricular, outflow tract, and atrial cardiomyocytes. The developmental trajectory of the human cardiac lineages broadly recapitulated that of the mouse, demonstrating conserved cardiovascular programs. Overall, our findings establish a comprehensive landscape of human embryonic cardiogenesis that provides access to a broad spectrum of cardiomyocytes for modeling congenital heart diseases and chamber-specific cardiomyopathies and for developing new therapies to treat them.

Funding Source: Canadian Institute of Health Research (CIHR FDN159937) funding from BlueRock Therapeutics LP
Keywords: cardiac mesoderm specification, generate distinct cardiomyocyte subtypes, single-cell transcriptome

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INVESTIGATION OF GENE-GENE INTERACTIONS FOR CONGENITAL HEART DISEASE SUSCEPTIBILITY USING HUMAN GENETICS

Lee, Kihyun - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Pittman, Maureen - *Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA*
Kayhani, Nilufar - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Pollard, Katherine - *Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA*
Srivastava, Deepak - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Identifying disease-causative genes has been a long-standing challenge, as the genetic components of disease are interwoven with many other factors that influence the disease susceptibility in human. To address this problem in congenital heart diseases (CHD), the Pediatric Cardiac Genomics Consortium has generated whole-exome sequencing data from CHD patients and their parents. Outflow tract (OFT) anomalies comprise more than 30% of CHD, and are life-threatening in the absence of surgical interventions. Therefore, we sought to identify the most causative genes for OFT anomalies by analyzing patients with truncus arteriosus (TA), the most severe form of OFT defects. We found that three TA patients harbored mutations in both of GATA6 and POR genes, despite diverse ethnic backgrounds. The affected regions of GATA6 and POR were located in different parts of protein, ruling out the possibility that the co-occurrence of mutations in multiple patients was caused by sequencing error. Previously, GATA6 mutations had previously been associated with OFT anomalies in humans with variable penetrance, leading to a broad range of phenotypes including normal development, implying that another genetic or environmental factors is needed to cause CHD in GATA6 mutation carriers. In mice, the deletion of POR, the cytochrome p450 oxidoreductase, causes early embryonic lethality due to defects in multiple organs including heart, suggesting that POR and GATA6 may interact during early development. Here, we show that bi-allelic deletion of Por in neural crest cells using Wnt1-cre mice (Por flox/del; Wnt1-cre) caused craniofacial defects, which was worsened upon Gata6 heterozygous deletion (Gata6 -/+; Por flox/del; Wnt1-cre), implying their genetic interactions. Directed differentiation of human pluripotent stem cells toward cardiac fates showed that POR modulates the levels of GATA6 expression via retinoic acid. Thus, this study demonstrates how gene-gene and gene-environment interactions can contribute to the disease susceptibility in humans.

Keywords: Congenital heart disease, Cardiac outflow tract, Genetic variants

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GENERATING INSULIN-RESISTANT ENGINEERED HEART TISSUE TO PROVIDE A HUMAN IN VITRO MODEL OF THE DIABETIC HEART FOR DISEASE MODELLING AND DRUG TESTING

Purnama, Ujang - *Department of Physiology, Anatomy, and Genetics, University of Oxford, UK*
Heather, Lisa - *Department of Physiology, Anatomy, and Genetics, University of Oxford, UK*
Carr, Carolyn - *Department of Physiology, Anatomy, and Genetics, University of Oxford, UK*

Abstract: Cardiovascular disease is the leading cause of mortality in diabetic patients. Understanding early stages of the disease is vital for seeking possible therapy targets. Despite numerous studies using in vitro and animal models, current preclinical models do not recapitulate the adult diabetic human heart. Therefore, there is a pressing need for an in vitro model to bridge the gap, to aid understanding of the factors of the diabetic phenotype that are harmful to cardiac tissue and to provide an accurate, predictive tool for drug testing. 3D culture of human induced-pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as engineered heart tissue (EHT) is the most advanced in vitro approach for cardiac disease modelling. Here, we show for the first time that modulating levels of glucose, insulin and palmitic acid in media for EHT will induce insulin resistance. After 6 days we observed a 26.7% decrease in glycolysis, a 25% increase in fatty acid metabolism and no increase in AKT phosphorylation in response to insulin, indicating that the hiPSC-CMs had become insulin resistant (IR-EHT). We subjected the IR-EHT to hypoxia and adrenergic stimulation and measured contractility using Muscle Motion. We saw a metabolic inflexibility of the cells under hypoxia which mimicked what we have observed in diabetic adult rat hearts. Treatment with Molidustat (BAY85-3934), a prolyl hydroxylase (PHD) inhibitor, rescued the impaired response to hypoxia by stabilising Hypoxia-Inducible Factor 1-α (HIF1α) signalling in hiPSC-CMs. Molidustat increased the expression of multiple genes involved in glycolysis caused twofold increase in glucose metabolism and

a 36% increase in lactate release, overriding inhibitory effect of insulin resistance in hypoxia. Treatment of IR-EHT with Metformin increased the sensitivity towards insulin stimulation and increased glucose consumption and lactate production, thereby confirming a direct effect of this drug on cardiomyocytes and validating its use as a therapeutic in the diabetic heart. Our results show that we have successfully generated a clinically relevant in vitro model of insulin-resistant human heart tissue to study the pathophysiological effects of diabetes. This can be a valuable resource to speed up the drug discovery process and improve candidate drug success in clinical trials.

Funding Source: Rosetrees Trust and Indonesia Endowment Fund for Education (LPDP)
Keywords: Diabetes, Engineered heart tissue, Drug testing

TOPIC: EARLY EMBRYO

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SYMMETRY BREAKING AND GERM LAYER EMERGENCE THROUGH OPTOGENETIC WNT ACTIVATION IN HUMAN PLURIPOTENT STEM CELL-DERIVED GASTRULOIDS

Johnson, Hunter J. - *Bioengineering, University of California, Berkeley, CA, USA*
Zimmermann, Joshua - *Chemical and Biomolecular Engineering, University of California, Berkeley, CA, USA*
Kim, Chang - *Anatomy and Psychiatry, University of California, San Francisco, CA, USA*
Repina, Nicole - *Bioengineering, University of California, Berkeley, CA, USA*
Bhalerao, Ritu - *Bioengineering, University of California, Berkeley, CA, USA*
Nowakowski, Tomasz - *Anatomy and Psychiatry, University of California, San Francisco, CA, USA*
Schaffer, David - *Bioengineering, Chemical and Biomolecular Engineering, Molecular and Cell Biology, University of California, Berkeley, CA, USA*

Abstract: In early embryogenesis, a series of intricately controlled fate decisions and patterning events during gastrulation establish the 3 germ layers and the initial 3D body plan of the developing organism. Spatiotemporal activation of morphogen signals regulates these dynamic developmental events and canonical Wnt signaling, in particular, is a critical vertebrate morphogen required for successful germ layer specification. However, due to technical and ethical constraints, a mechanistic understanding of Wnt signaling in human development is severely limited. Human pluripotent stem cells (hPSCs) offer the potential to model and understand key human developmental events, but present gastrulation models are limited by lack of spatiotemporal control of signal presentation in vitro. The specific role of asymmetric Wnt activation in specifying germ layer formation, as well as cellular organization within hPSC-derived gastruloid models has yet to be determined. To address these limitations, we have recently engineered an optogenetic toolkit that enables precise spatiotemporal control of canonical Wnt signal activation in hPSCs (optoWnt) using only blue light illumination. To model selective Wnt activation in 3D gastruloids, we aggregated optoWnt hPSCs with wild type (wt) hPSCs at a 50:50 ratio under continuous blue light illumination, such that only the optoWnt cells respond with Wnt activation in a salt-and-pepper distribution. After 60-hours of selective optoWnt activation, mixed hPSC aggregates observed a symmetry breaking event, with segregation of the optoWnt hPSCs from the wt-hPSCs, and emergence of cell patterning resembling that of the 3-germ layers. Specifically, the wt-hPSCs exhibited markers of the ectoderm while the optoWnt-hESCs specified to either mesoderm or endoderm, as characterized by ICC, RT-qPCR and scRNAseq. We linked the symmetry breaking and cellular segregation to E-cadherin/N-cadherin switching, following an epithelial to mesenchymal transition. Finally, we demonstrate that the continued activation of TGFβ within the optoWnt cells induces endoderm, while transient TGFβ signaling is necessary for mesoderm specification over the 60-hour period. Our findings support the role of selective Wnt signaling in hPSC symmetry breaking and germ layer specification.

Funding Source: US National Science Foundation (to H.J.J. and N.A.R.) and US National Institutes of Health R01NS087253 (to D.V.S.)
Keywords: Optogenetics, Gastrulation, Gastruloids

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MOUSE TROPHOBLAST CELLS ARE INSENSITIVE TO THE CYTOTOXICITY OF TUMOR NECROSIS FACTOR-ALPHA AND INTERFERON-GAMMA

Fendereski, Mona - *Cell and Molecular Biology, University of Southern Mississippi, Hattiesburg, MS, USA*
Bae, Yeseul - *Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, USA*
Guo, YanLin - *Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, USA*

Abstract: An embryo faces many immunological challenges during early embryogenesis. At the implantation site, the embryo is exposed to cytokines that are necessary for implantation but also negatively affecting cell proliferation and viability. TNFα and IFN*γ* are two such inflammatory cytokines. In particular, their combination (TNFα/IFNγ) can cause cell death to many types of cells. How an early embryo can avoid immunologic cytotoxicity is a fundamental yet poorly understood question. We investigated the effects of TNFα and IFN*γ* on three main cell types in the blastocyst (the preimplantation embryo): embryonic stem cells (ESCs), trophoblast stem cells (TSCs), and in-vitro differentiated trophoblasts (TSC-TBs). We recently reported that TNFα/IFNγ effectively kill fibroblasts, in part by, induction of inducible nitric oxide synthase (iNOS). Remarkably, this treatment has no apparent effect on ESCs due to their lack of ability to express iNOS. However, ESCs are sensitive to nitric oxide (NO) chemically released from sodium nitroprusside (SNP) in the cell culture, suggesting that NO can be detrimental for ESCs in the blastocyst cavity if excessive amount is released from TSCs and TSC-TBs. Interestingly, our data revealed that, like ESCs, TSCs and TSC-TBs are insensitive to the cytotoxicity of TNFα/IFNγ, which could also be due to the lack of iNOS expression. To test this hypothesis, we compared the responses of TSCs and TSC-TBs and fibroblasts to TNFα, IFNγ, and TNFα/IFNγ. We demonstrate that TNFα, IFN*γ* and TNFα/IFNγ induced robust expression of multiple genes in fibroblast, including iNOS. However, TSCs and TSC-TBs showed limited responses and failed to express iNOS. It is known that TNFα and IFN*γ* induced iNOS requires synergistic activation of NFκB and STAT1 pathways. IFN*γ* weakly activates STAT1 in TSCs and TSC-TBs but TNFα fail to activate NFκB, which together explain the lack of induction of iNOS in these cells. Furthermore, TSC-TBs are less sensitive to the cytotoxicity of NO released by SNP. These findings suggest that TSCs and TSC-TBs have attenuated responses to TNFα and FN*γ*, which may serve as a protec-

tive mechanism that minimizes the inflammatory toxicity to the blastocyst during implantation.

Keywords: Mouse trophoblast stem cells, Tumor necrosis factor-α (TNFα), Inducible nitric oxide synthase (iNOS)

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LHX1 ANCHORED GENE REGULATORY NETWORK ENCOMPASSES NOVEL HEAD ORGANISER TRANSCRIPTION FACTORS

McMahon, Riley - *Embryology Research Unit, Children's Medical Research Institute, Westmead, New South Wales, Australia*
Sibbritt, Tennille - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Graham, Tamara - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Aryamanesh, Nader - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Masamsetti, V. Pragathi - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Tam, Patrick P.L. - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*

Abstract: Embryonic development is driven by molecular instructions encoded by transcription factors (TFs) that underpin the formation of the body plan and the specialisation of tissue precursor cells. Analysis of gastrulating mouse embryos has revealed that the LIM homeobox 1 (LHX1) TF is indispensable for head and face development. However, the precise function of LHX1 in the initiation of craniofacial morphogenesis at late gastrulation has not been fully elucidated. Here we present an LHX1 anchored gene regulatory network in embryos utilising multi-omics analytics including RNA-seq, ATAC-seq and DamID-seq. We identified the forkhead box gene, Foxd4 and the BTB domain gene, Kctd1, as direct downstream targets of LHX1. CRISPR-Cas9 edited mouse embryonic stem cell (mESC) lines were generated with bi-allelic frameshift mutations in the coding region of these two target genes. The function of these TFs was investigated using chimeric embryos harbouring the gene-edited mESCs and the stem cell-derived neuruloid model. We showed that FOXD4 is essential for neurulation in the rostral neural tube and for the specification of the cranial neural crest population and the loss of KCTD1 activity that impacted the canonical Wnt signalling pathway de-railed mesendoderm lineage development. Overall, our findings have highlighted the role of these LHX1 targets in the development of the head and face, which are major body parts of the early mammalian embryo.

Keywords: gene regulatory network, head development, neural tube defects

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DIFFERENTIAL RESPONSES TO MECHANOSTIMULATION IN EMBRYONIC STEM CELLS VERSUS THE EMBRYOID BODY MODEL OF DEVELOPMENT ASSESSED AT SINGLE CELL RNA-SEQ RESOLUTION

Juran, Cassandra M. - *Space Biology, NASA Ames Research Center, Palm City, CA, USA*
Almeida, Eduardo - *Space Biology, NASA, Mountain View, CA, USA*

Abstract: Mechanical forces generated by gravity have shaped life on Earth and impact gene expression and morphogenesis during early development. In contrast disuse can reduce normal mechanical loading, resulting in altered cell and tissue function. Although loading in adult mammals is known to promote increased cell proliferation and differentiation, little is known about how cells respond to this stimulus during early development. In this study we sought to understand, with single cell RNA-sequencing resolution, how a 60-minute pulse of 50xg hypergravity-generated 5kPa hydrostatic pressure, influences transcriptomic regulation of developmental processes in the Embryoid Body (EB) model. Our study included both day-9 EBs and progenitor mouse embryonic stem cells (ESCs) with or without the hydrostatic pressure pulse. Single cell tSNE mapping shows limited transcriptome shifts in response to this pulse in either ESCs or EBs; this pulse, however, induces greater positional shifts in EB mapping compared to ESCs, indicating the influence of mechanotransduction is more pronounced in later states of cell commitment within the developmental program. We assessed ESCs and EBs for differentially expressed (DE) genes with hydrostatic pressure pulse and found approximately 1/3 DE genes were shared. However, gene ontology (GO) pathway analysis show that EBs have choreographed responses associated with upregulation of pathways for multicellular development, mechanical signal transduction, and DNA damage repair. Cluster transcriptome analysis of the EBs shows mechanostimulation promotes maintenance of transitory cell phenotypes in early development, including EB cluster co-expression of markers for progenitor, post-implant epiblast and primitive endoderm phenotypes versus expression exclusivity in the non-pulsed clusters. Pseudotime analysis identified three branching cell types susceptible to hydrostatic pressure induction of cell fate decisions. In summary, this study provides novel evidence that ESC maintenance and EB development can be regulated by mechanostimulation, and that stem cells committed to a differentiation program are more sensitive to force-induced changes to their transcriptome.

Funding Source: This work was supported by a NASA Space Biology grant NNH14ZTT001N-0063 to Dr. E. Almeida, and a NASA Space Biology Postdoctoral Fellowship to Dr. C. Juran.
Keywords: mechanotransduction, embryonic stem cells, single cell RNA sequencing

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A COMBINATORIAL EFFECT BETWEEN BMP AND WNT SIGNALING HIGHLIGHTS THE IMPORTANCE OF TIME IN HUMAN EMBRYONIC STEM CELL DIFFERENTIATION

Camacho Aguilar, Elena - *Biosciences, Rice University, Houston, TX, USA*
Yoon, Sumin - *Biosciences, Rice University, Houston, TX, USA*
Warmflash, Aryeh - *Biosciences, Bioengineering, Rice University, Houston, TX, USA*

Abstract: Secreted morphogen signals play a key role in the determination of cell fates during embryonic development. In particular, BMP signal is essential for vertebrate gastrulation, as it initiates the self-organized patterning of the three germ layers. Although morphogen signals are typically thought to induce cell fates in a concentration-dependent manner, development is a highly dynamic process, so it is crucial to understand how time-dependent signaling affects cellular differentiation. Recent studies have suggested that duration and amplitude of signal may be interchangeable, with longer exposure to low doses be-

ing equivalent to shorter treatment with high doses. We find that this is not the case for BMP mediated differentiation in human embryonic stem cells (hESCs). Instead, varying the duration of BMP signaling leads cells to transit through pluripotent, mesodermal, and extraembryonic states, while varying the concentration does not cause efficient mesodermal differentiation at any dose. Thus, there is a morphogen effect in time but not in concentration, and an appropriately timed pulse of BMP signal induces hESCs to a mesodermal fate much more efficiently than sustained signaling at any concentration. Using live cell imaging of signaling and cell fate reporters together with a simple mathematical model, we show that this effect is due to a combinatorial interpretation of the applied BMP signal and endogenous WNT signaling. Our findings have implications for how signaling pathways control the landscape of early human development.

Funding Source: This work was funded by Rice University and grants to AW from NSF (MCB-1553228, MCB-2135296), NIH (R01GM126122), Welch Foundation (C-2021), and Simons Foundation (511079).
Keywords: human gastrulation, cell fate transitions, signal interpretation

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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MECHANISMS IN REVERSING DYSFUNCTION IN THE AGING BRAIN VASCULATURE

Florido, Mary Heather Celine C. - *Harvard Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Environmental Health Department, John B. Little Radiation Center, Harvard University, Harvard T.H. Chan School of Public Health, Cambridge, MA, USA*
Hilton, Kylie - *Molecular and Cellular Biology, Harvard Stem Cell and Regenerative Biology Department, Harvard University, Cambridge, MA, USA*
Spetz, Johan - *Environmental Health Department, John B. Little Radiation Center, Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA, USA*
Sarosiek, Kristopher - *Environmental Health Department, John B. Little Radiation Center, Harvard T.H. Chan School of Public Health, Boston, MA, USA*
Rubin, Lee - *Harvard Stem Cell and Regenerative Biology Department, Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA*

Abstract: The blood-brain-barrier (BBB) serves as a tight gatekeeper between the brain and the rest of the body. Brain endothelial cells (ECs), a cellular component of the BBB, are a highly specialized type of endothelia with low rates of transcytosis and high-resistance tight junctions that combine to control the movement and transport of substances into and out of the brain. In several diseases, as well as during aging, the BBB is altered, thereby contributing to the progression of neurodegenerative phenotypes. Despite the importance of this structure and its involvement in various neurodegenerative diseases, it remains heavily understudied. We hypothesize that as we age, brain endothelial cell fitness declines and is accompanied by metabolic changes, ultimately leading to frank loss of vasculature as a result of increased apoptosis. To support this hypothesis, we have previously generated a single-cell RNA sequencing dataset comparing young versus old mouse brains and, in fact, observed a number of dysregulated pathways, including key metabolic pathways. In addition, we performed BH3 profiling assays of brain ECs and determined that they are highly primed to undergo cell death in response to various damaging stimuli. We also confirmed differential apoptotic gene expression between peripheral and brain ECs using various published RNA-seq datasets. Apoptosis is a mitochondria-driven process, and, excitingly, we also found changes in genes associated with glycolysis and oxidative phosphorylation. We next performed metabolomic and lipidomic studies on young and old mouse brain ECs, confirming differential abundance in a number of metabolites. Taken together, our data show that brain ECs are primed for apoptosis compared to other cell types and that their metabolism is altered in aging. In the future, we aim to modulate mitochondrial metabolism or prevent apoptosis to reverse the dysfunction in aging brain vasculature and thereby identify potential therapeutic targets.

Funding Source: This research is supported by the following NIH Grants: NINDS (1R01NS117407) and NIH R37 grant (R37CA248565-01).
Keywords: Aging in the brain vasculature, brain endothelial cells, cell death

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A HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF NILOTINIB-INDUCED ARTERIAL DISEASE

Pinheiro, Emily - *Department of Pharmacology, Northwestern University, Chicago, IL, USA*
Burridge, Paul - *Department of Pharmacology, Northwestern University, Chicago, IL, USA*

Abstract: Nilotinib is a highly effective tyrosine kinase inhibitor used to treat Chronic Myeloid Leukemia (CML). While nilotinib successfully treats CML, a common and serious side effect is development of nilotinib-induced arterial disease (NAD) even in patients without pre-existing risk factors. This side effect is not seen with imatinib, a CML drug in the same class that inhibits the same target as nilotinib. To date, the mechanisms of NAD are unknown, and the cell types involved in this adverse effect have yet to be identified. Furthermore, no tools currently exist to predict which patients will develop NAD. In this study, we used endothelial and vascular smooth cells derived from human-induced pluripotent stem cells (hiPSCs) as a model to identify which cell types are involved in NAD and which cellular functions are perturbed. We first developed a set of efficient, reproducible endothelial and vascular smooth muscle differentiation and purification protocols that consistently yield >95% pure cells. For both hiPSC-derived endothelial cells (hiPSC-ECs) and hiPSC-derived vascular smooth muscle cells (hiPSC-VSMCs) we next identified a panel of assays relevant to various functions of atherogenesis. In comparing the effects of nilotinib and imatinib on hiPSC-ECs, we see a nilotinib-specific decrease in cell capacity for proliferation and migration, in addition to disturbances in Ac-LDL uptake and NO homeostasis. Nilotinib is not differentially toxic to hiPSC-ECs compared to imatinib and has no effect on barrier function as measured by multielectrode array. The data collected to date demonstrate the feasibility of using hiPSCs to model NAD in vitro and recapitulate the clinical discrepancy in vascular effects between nilotinib and imatinib.

Funding Source: 1F31HL151160
Keywords: Vascular, Nilotinib, Arterial Disease

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TOWARDS HIGH EFFICIENT GENERATION OF ALVEOLAR TYPE 2 EPITHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Rodriguez Ruiz, Alejandro - *Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden University Medical Center, Leiden, Netherlands*
Ravi, Abilash - *Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden University Medical Centre, Leiden, Netherlands*
Khoenkhoen, Shewanti - *Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden University Medical Centre, Leiden, Netherlands*
Dias Brescia, Marcella - *Department of Anatomy, LUMC, Leiden University Medical Centre, Leiden, Netherlands*
van Meer, Berend - *Department of Anatomy, LUMC, Leiden University Medical Centre, Leiden, Netherlands*
Olmer, Ruth - *Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany, Hannover Medical School, Hannover, Germany*
Martin, Ulrich - *Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany, Hannover Medical School, Hannover, Germany*
Hiemstra, Pieter - *Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden University Medical Centre, Leiden, Netherlands*
van der Does, Anne - *Department of Pulmonology, Leiden University Medical Center (LUMC), Leiden University Medical Centre, Leiden, Netherlands*

Abstract: Progressive alveolar damage (emphysema) is a hallmark of patients with chronic obstructive pulmonary disease (COPD). COPD is the third leading cause of death worldwide and is expected to remain a major health problem because of smoking, the aging population and air pollution, while there are no current treatments available. Human induced pluripotent stem cells (hiPSCs) are increasingly used for generation of alveolar type 2 epithelial cells (iAEC2) to study alveolar injury and regeneration. The differentiation process is however still inefficient. The aim of the present study was to increase efficiency of iAEC2 generation from hiPSC. Using an hiPSC NKX2.1-GFP reporter line, the impact of several compounds, cell seeding densities, and gel matrixes on several steps of the differentiation protocol toward the NKX2.1+ lung progenitor stage was evaluated using gene expression, immunohistochemistry, flow cytometry and Incucyte® live-cell imaging analyses. A cell passage ratio of 1:12 in hiPSC-derived definitive endoderm (DE) cells combined with a vitronectin-coated surface, showed superior generation of anterior foregut endoderm (AFE) cells when compared to using a higher cell density and other coatings. Selected and combined administration of the BMP inhibitors dorsomorphin and noggin was compared to further improve AFE differentiation, together with the TGFβ-inhibitor SB-431542. Results demonstrated higher efficiency in generating FOXA2+/ SOX17- AFE cells for dorsomorphin (85.9%) than noggin (47.1%) or when combined (59.5%). Live imaging during hiPSC differentiation revealed that in control cultures, ~7% of ventralized AFE cells expressed NKX2.1 GFP. Future experiments will use live imaging to read-out strategies to further increase AFE to VAFE differentiation. We demonstrated that vitronectin-coated surfaces combined with a 1:12 cell passage ratio, together with SB-431542 and dorsomorphin are essential for optimizing generation of the AFE lineage. Moreover, we showed the feasibility of real-time tracking of NKX2.1 expression using a reporter cell line combined with live imaging strategies for subsequent steps in protocol optimization.

Funding Source: This study was supported by Health Holland (LSHM20104), Longfonds Nederlands (Project number 4.1.19.021) and LSHM20018 (Stichting Proefdiervrij and Sartorius Stedim Biotech GmbH). The study is part of the P4O2 consortium.
Keywords: lung, epithelial cells, hiPSCs

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INVESTIGATING RESPIRATORY SYNCYTIAL VIRUS INFECTION IN BIOLOGICALLY RELEVANT, INDUCED PLURIPOTENT STEM CELL DERIVED CO-CULTURE MODEL SYSTEMS.

Lovering, Frank M. - *College of Sciences, San Diego State University, Coronado, CA, USA*
McVicar, Rachael - *SPB, Sanford Burnham Prebys, San Diego, CA, USA*

Abstract: Respiratory Syncytial Virus (RSV) is a ubiquitous pathogen that causes an extreme disease burden across the globe. RSV infects an estimated 64 million annually in addition to causing 160,000 deaths. Nearly every child is infected with RSV by the age of two, and it is the leading cause of pneumonia in neonates. Premature birth, bronchopulmonary dysplasia, and genetic polymorphisms are all known comorbidities for this disease. A compounding factor in the aforementioned comorbidities is a deficiency in pulmonary surfactant. Surfactant, a viscous mixture of proteins and lipids that lines the inner lung, reduces alveolar surface tension and contributes to host antimicrobial defense. Deficiencies in the surfactant proteins can render the fluid ineffective. Our research focuses on surfactant protein B (SP-B). SP-B deficiency is associated with severe RSV outcomes, but the mechanism behind this association is still unknown. Utilizing both healthy and SP-B mutant human induced pluripotent stem cells (hiPSCs), our lab has generated 3-D lung models to investigate the role this protein plays in regulating viral clearance. To increase the biological relevance of our models, immune cells will be co-cultured with lung epithelial cells. Differentiating immune cells and lung epithelial cells from patient specific cell lines will maintain genetic consistency in our model systems. Preliminary data has shown that our directed lung differentiations produce the cell types present in the human lung. Robust expression of markers for lung basal cells, club cells, goblet cells, alveolar type I cells, and alveolar type II cells has been detected. To our knowledge, SP-B mutant hiPSC cell lines have never been used to generate lung/immune cell co-cultures, and the results obtained from infection experiments in these models will be novel to the scientific community.

Keywords: Virus, Surfactant, Macrophages

TOPIC: EPITHELIAL_SKIN

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SKIN ORGANOID AS A UNIVERSAL PLATFORM FOR SKIN PHYSIOLOGY AND DISEASE MODELING

Gorkun, Anastasiya - *Atala's group, Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA*
Atala, Anthony - *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA*
Gironda, Daniel - *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA*
Jorgensen, Adam - *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA*
Mahajan, Naresh - *Atala's group, Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA*
Soker, Shay - *Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC, USA*
Wu, Mingsong - *Higher Education Institution of Guizhou Province, Zunyi Medical University, Winston-Salem, NC, USA*

Abstract: Tissue organoids are proposed as in vitro models for drug testing, organ development, and disease. In this study, we applied healthy skin organoids (SO) as a universal platform to model skin tissue development, skin physiology, chemical irritation, effects of UV radiation, and skin cancer. Human skin cells: keratinocytes, melanocytes, fibroblasts, endothelial cells, follicle dermal papillae cells, and adipocytes were mixed to form SOs and their anatomy and pigmentation were characterized for up to 21 days in culture. Normal skin physiology and barrier function were tested with retinol metabolism and Lucifer Yellow diffusion, respectively. For radiation modeling, SOs were exposed to UVB (150mj/cm2). For chemical irritation testing, SOs were incubated with 1% Triton for 6h, and with Isopropanol, Hexyl Salicylate, and 5% KOH for 15min on day 7 of culture. For disease modeling, SK-MEL-28, a melanoma cell line was either incorporated with the normal skin cells during SOs formation or added on day 7. The melanoma SOs were analyzed using histology, immunohistochemistry, cell viability assays, and RT-PCR. SOs demonstrated skin-like layered microstructure, with the surface zone formed by epidermis cells and the central core formed by dermal and hypodermal cells and primitive vascular structures. SOs showed skin-like functionality through the metabolism of retinol into retinoic acid, active melanogenesis, and epidermal barrier formation. After exposure to UVB, the SOs demonstrated ER-stress and apoptosis. SOs maintained barrier integrity against 1% Triton over 2h with further separation of the surface and the core, and reduction in cell viability. Isopropanol and Hexyl Salicylate were non-irritants, with cell survival >50%, while highly irritant 5% KOH lowered cell viability to 20%. Modeling of melanoma with SK-MEL-28 cells showed tumor cell proliferation inside and on the surface of the SO, resembling metastasis. In conclusion, the current study shows that the multicellular SOs are capable of recapitulating skin anatomy and function, allowing them to serve for high-throughput analysis under immersive conditions. Ultimately, the SO technology can provide an in-vitro skin model that could be used as a platform for dermatopathological and cancer biology research and drug development.

Keywords: Skin organoid, Chemical irritation testing, Melanoma modeling

TOPIC: EYE AND RETINA

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PRB-DEPLETED PLURIPOTENT STEM CELL RETINAL ORGANOIDS AS A 3D IN VITRO MODEL FOR DRUG SCREENING IN RETINOBLASTOMA

Cerna Chavez, Rodrigo - *Biosciences Institute, Newcastle University, Newcastle Upon Tyne, UK*
Rozanska, Agata - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
Zerti, Darin - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
Dorgau, Birthe - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
Steel, David - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
Benvenisty, Nissim - *The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel*
Armstrong, Lyle - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*
Parulekar, Manoj - *Ophthalmology Department, Birmingham Women's and Children's Hospitals, Birmingham, UK*
Lako, Majlinda - *Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK*

Abstract: Retinoblastoma (Rb) is a retinal tumour affecting infants younger than five years with a prevalence of 1:15,000 that can cause death if left untreated. The biallelic inactivation of the RB1 tumour suppressor gene accounts for up to 98% of Rb cases. Regardless of treatment progress, Rb survivors have vision impairments that impact accurate motion processing, depth perception, and distance judging. Hearing loss, cataracts, physical abnormalities, and delayed neurocognitive development are all possible side effects that patients can suffer. As fundamental tools and platforms are needed for 3D in vitro retinal disease modelling, we developed and characterised two in vitro Rb models for drug screening and testing. We generated retinal organoids derived from an RB1 knock-out human embryonic stem cell (hESC) line. Then, we developed retinal organoids from a patient-specific induced pluripotent stem cell (iPSC) model (c.2082delC), including the homozygous (RB1-/-) and the heterozygous (RB1+/-) mutants, and fully corrected isogenic control (RB1+/+). To validate their application for drug screening, we tested the impact of three clinically-used chemotherapeutic agents: melphalan, topotecan and TW-37. We compared our established iPSC and hESC models performing immunofluorescence, lactate dehydrogenase release, cell-cycle and colony formation analysis. Both organoid models showed key features of tumorigenesis, such as an increased fraction of proliferating cone precursors (RXRγ+Ki67+), proliferating (Ki67+) and apoptotic cells (Caspase-3+), and cell growth in an anchorage-independent manner in soft agar assays of pRB-depleted organoids. The application of melphalan, topotecan, and TW-37 resulted in a significant decrease in Rb proliferating cone precursors and a significant increase in the fraction of apoptotic cone precursors specifically in the Rb models but not controls, indicating these in vitro models are suitable for evaluating novel Rb treatments. In conclusion, we have developed and fully characterised an hESC and a patient-specific iPSC Rb retinal organoid model and have demonstrated that these accurately reproduce

Rb development in vivo, providing a powerful platform for pharmacological and toxicological testing.

Funding Source: CHECT, Fight for Sight, and CONACYT.
Keywords: Human induced pluripotent stem cells, Retinoblastoma, Retinal organoids

TOPIC: GERMLINE

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EMBRYONIC AND FETAL TESTICULAR DEVELOPMENT OF THE RHESUS MACAQUE

Sosa, Enrique - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, West Hills, CA, USA*
Hsu, Fei-Man - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Wamaitha, Sissy - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Clark, Amander - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*

Abstract: The fetal period of testicular development remains poorly understood in humans. The non-human primate, Rhesus macaque, serves as an important pre-clinical model that allows us to explore critical developmental timepoints that otherwise would be inaccessible due to ethical concerns and limitations. To address this, we used a time-mated breeding strategy to study a period of embryonic development that encompasses the end of organogenesis (Carnegie stage 23-Embryonic Day 50) and spans the second and third trimesters of fetal life, all complex steps necessary to undergo faithful recapitulation of testicular development in an in vitro context. Using 10x Single Cell Genomic Sequencing technology during this critical window of testicular development we have identified discrete populations of cells that correspond to major players of the testis including Sertoli, Leydig, immune, as well as interstitial/mesenchymal cells. Using histological methods, we found that there are several gross anatomical changes that occur from day 50 to day 130. The most apparent is the thickening of the capsule from day 50 onwards as well as the sex-specific changes that occur during sex cord to testis cord formation. During this fetal period there is apparent tubule lumen formation as early as D100, which is earlier than reported for the mouse and human testes. Immunohistochemistry staining revealed that the number of DDX4+ germ cells that express germ cell specifier genes such as TFAP2C or SOX17 are all but gone by day 130. Almost immediately after D100, DDX4+ germ cells begin to extinguish pluripotency gene expression (i.e. NANOG). Conversely, as the naïve pluripotency program is lost the State F0 program (i.e. PIWIL4) is upregulated in DDX4+ germ cells. Using the non-human primate as a model is critical for our understanding of the cellular players and key events involved during testes development. Insight from these studies may be useful to successfully recapitulate testicular development, as well as gametogenesis in vitro.

Keywords: Testes development, Germ cells, non-human primate

TOPIC: HEMATOPOIETIC SYSTEM

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INVESTIGATING THE ROLE OF KLF1 IN PRIMITIVE AND DEFINITIVE ERYTHROPOIESIS

Pavani, Giulia - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA, USA*
Klein, Joshua - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA, USA*
Nations, Catriana - *Department of Cell and Molecular Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA*
Brooks, Dominique - *Department of Cell and Molecular Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA*
An, Hyun Hyung - *Pediatrics, Children's Hospital of Philadelphia, PA, USA*
Abdulmalik, Osheiza - *Pediatrics, Children's Hospital of Philadelphia, PA, USA*
Chou, Stella - *Pediatrics, Children's Hospital of Philadelphia, PA, USA*
French, Deborah - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA, USA*
Gadue, Paul - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA, USA*

Abstract: At least two hematopoietic waves generate erythroid progenitors during development: primitive, which originates in the yolk sac, and definitive, which originates from large vessels and colonizes the fetal liver (FL). Primitive red blood cells (RBCs) are larger, nucleated, and express embryonic globins, while definitive RBC are smaller, enucleated and contain fetal globins. KLF1 is a transcription factor that regulates erythroid cell development. Mouse studies have shown increased levels of KLF1 in the FL versus yolk sac, suggesting differential KLF1 requirements during primitive and definitive erythropoiesis. To study KLF1 during primitive and definitive hematopoiesis in a human model system, induced pluripotent stem cells (iPSCs) were generated from a human FL sample. Using established in vitro differentiation protocols that produce either primitive or definitive hematopoietic progenitors, RBCs were generated from the iPSC line and compared to isogenic RBCs generated directly from the FL sample. Analysis by sc-RNAseq showed comparable gene expression patterns for the definitive RBCs generated from iPSCs and FL cells and a distinct gene expression pattern for the iPSC-derived primitive RBCs. Using base editors, the iPSC line was genome edited to introduce a known KLF1 pathogenic variant in the DNA-binding domain (L300P) or an early stop codon (Q24X) and presumed null allele. Homozygous loss of KLF1 with either variant led to limited expansion, lack of hemoglobinization, and no/low expression of red cell markers using the primitive differentiation protocol. Heterozygous lines were partially impaired, with the L300P variant displaying a more severe defect compared to Q24X, suggesting a dominant negative effect. RNA-seq analysis showed that KLF1 variants impacted cell cycle and ribosomal genes, while inducing megakaryocytic genes. Using the definitive differentiation protocol, we observed a relative increase in expression of embryonic globins at the expense of fetal globins in L300P/+ RBCs while globin composition was unaffected in primitive RBCs. These studies provide new insights into how the primitive and definitive programs differ regarding their utilization of KLF1 and highlight the

importance of the differentiation program when using iPSCs to model genetic hematologic diseases.

Keywords: Erythropoiesis, Fetal liver hematopoiesis, Red blood cells

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AN EX VIVO LIVER MICROENVIRONMENT THAT SUPPORTS HEMATOPOIETIC STEM CELL MAINTENANCE AND EXPANSION

Paik, Na Yoon - *Pharmacology, University of Illinois at Chicago, IL, USA*
Brown, Grace - *Department of Bioengineering, University of Illinois at Chicago, IL, USA*
Sottoriva, Kilian - *Department of Pharmacology, University of Illinois at Chicago, IL, USA*
Shao, Lijian - *Department of Pharmacology, University of Illinois at Chicago, IL, USA*
Khetani, Salman - *Department of Bioengineering, University of Illinois at Chicago, IL, USA*
Pajcini, Kostandin - *Department of Pharmacology, University of Illinois at Chicago, IL, USA*

Abstract: Every year, 18,000 people require bone marrow transplants to treat hematologic malignancies and diseases according to the US Department of Health and Human Services. While hematopoietic stem cells (HSCs) have high therapeutic value in treating hematologic malignancies, the low number of HSCs obtainable for autologous transplants and the difficulty in finding matching HLA donors are the critical limitations of this treatment. In addition, allogenic transplantations may lead to complications of graft rejection. Hence, a system that can expand cord blood or bone marrow HSCs ex vivo would greatly improve the clinical applications of bone marrow transplants. While the primary site of hematopoiesis is the bone marrow, the liver is also a main site of hematopoiesis during development and in pathologic conditions. The fetal liver is the main site of HSC expansion during development, whereas the adult liver is a temporary site of HSC homing when the bone marrow is damaged. Our goal is to create a culture system that mimics the liver hematopoietic microenvironment. We have bioengineered a coculture system that consists of primary human hepatocytes (PHHs) and 3T3-J2 mouse fibroblasts. Onto this coculture, we cultured hematopoietic stem and progenitor cells (HSPCs) in serum-free medium supplemented with pro-hematopoietic cytokines. HSPCs were cultured on this PHH+J2 microenvironment for two weeks and show over 200-fold expansion and formed tight clusters around the periphery of the PHH islands. These expanded cells retained the HSPC phenotypic markers of Lin-, Sca1+, cKit+, as well as the long-term phenotypic HSC SLAM markers. In addition, the ex vivo expanded cells were transplanted and showed serial reconstitution potential of lethally irradiated recipient mice. Expanding on this coculture system, we are currently expanding human CD34+ cord blood cells in a human liver microenvironment platform to understand the niche factors that contribute to maintenance and expansion for therapeutic applications.

Keywords: hematopoietic stem cell, ex vivo liver niche, hematopoietic stem cell expansion

TOPIC: IMMUNE SYSTEM

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GENETICALLY ENGINEERED DENDRITIC CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS TO ACTIVATE SARS-COV-2 PROTEIN-SPECIFIC T-CELLS TO TREAT COVID-19

Begum, Aynun N. - *Biomedical Science Division, NeyroblastGX LLC, Murrieta, CA, USA*
Michkov, Alexander - *Biomedical Science Division, NeyroblastGX LLC, Murrieta, CA, USA*

Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a significant risk for vaccinated and non-vaccinated populations. The SARS-CoV-2 virus is constantly mutating, having the potential to cause serious harm to the global population. The highly contiguous Omicron variant is the dominant strain in the US, infecting 2000 people/day. According to the CDC (February 02, 2022), probable cases of COVID-19 stand at 75 million, and estimated deaths exceed 884,800 in the US. Severe COVID-19 or lingering symptoms of long COVID patients possess significantly reduced numbers of endogenous CD4+ and CD8+ T cells. No universal T cell therapy exists to boost T cell production in older, immunocompromised, and severe COVID-19 or long COVID patients to prevent breakthrough infections from newly emerging COVID-19 variants. NeyroblastGX LLC (NGL) aims to develop genetically engineered human induced pluripotent stem cells (hiPSC) derived dendritic cells (DCs) as a probe transfected with various SARS-CoV-2 proteins (e.g., spike, nucleocapsid, membrane). This DC-COV19 probe will be used to produce high functional SARS-CoV-2-specific universal CD4+, and CD8+ T cells in cGMP (current good manufacturing practices) grade ex vivo. These autologous immune T cells will then be transferred into COVID-19 patients as a rapid and robust adaptive T cell-based immunotherapy. NGL generated DCs under cGMP condition on a robust scale, which highly expressed DC-Sign, CD40, CD86, CD11C, MHC Class II protein, and HLA-DRA determined via flow cytometry and immunocytochemistry. We transfected our DC with various epitopes of SARS-CoV-2 proteins with >80% efficiency. Our multi-epitope engineered DCs (eDCs) were able to significantly activate SARS-CoV-2 specific T cells. NGL works with world-class immunologists on the feasibility of this system to develop SARS-CoV-2 multi-epitope constructs for use in eDCs to activate SARS-CoV-2 universal CD4+ and CD8+ immunotherapeutic T cells for COVID-19.

Funding Source: This work is supported by National Science Foundation (NSF) COVID-19 initiative.
Keywords: Genetically engineered dendritic cells, COVID-19 and long COVID, Universal T cells therapy

TOPIC: KIDNEY

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IDENTIFYING NOVEL THERAPEUTIC TARGETS AND BIOMARKERS FOR CHRONIC KIDNEY DISEASE USING HIPSC-DERIVED PODOCYTES

Burt, Morgan A. - *Biomedical Engineering, Duke University, Durham, NC, USA*
Mehta, Nalini - *Biomedical Engineering, Duke University, Durham, NC, USA*

Bhattacharya, Rohan - *Biomedical Engineering, Duke Univerity, Durham, NC, USA*
Kalejaiye, Titilola - *Biomedical Engineering, Duke Univerity, Durham, NC, USA*
Dimitrakakis, Nikolaos - *Biologically Inspired Engineering, Wyss Institute, Harvard University, Boston, MA, USA*
Okafor, Arinze - *Cell Biology, Duke Univerity, Durham, NC, USA*
Musah, Samira - *Biomedical Engineering, Nephrology, Duke University, Duke Medical School, Durham, NC, USA*

Abstract: The development of physiologically relevant, human stem-cell derived models is poised to provide molecular-level insight into the pathology of the most medically recalcitrant human diseases. Here, we demonstrate how human induced pluripotent stem cell (hiPSC)-derived podocytes enable the identification of potential early biomarkers and therapeutic targets to diagnose and treat chronic kidney disease (CKD). CKD is a degenerative disorder estimated to affect 15% of the global population, and there are no targeted therapies. The most severe forms of CKD stem from irreversible damage to the glomerular podocytes, the highly specialized, post-mitotic epithelial cells that help filter the blood. Historically, modeling human podocytopathies in vitro has been difficult due to the lack of reliable, primary cell models. Recent findings from human kidney biopsies suggest that abnormal podocyte biomechanics, specifically the dysregulation of the podocyte actin cytoskeleton, is a common pathological feature in proteinuric diseases, despite etiological diversity. However, it is not clear how podocyte biomechanics influence cell fate decisions in the context of the injured glomerulus. To address this issue, we employed our method of deriving mature podocytes from hiPSCs to examine the activity of mechanosensitive proteins in response to various clinically relevant injury modalities, including renal inflammation (lipopolysaccharide-induced), drug-induced nephropathy (Adriamycin), diabetic nephropathy, and disruption of cytoskeletal integrity (cytochalasin D-induced). We demonstrate that we can use these hiPSC-derived podocytes to mimic the aberrant cytoskeletal remodeling observed in the podocytes of CKD patients. Additionally, we show how performing omics-level analyses on multiple disease models enables us to systematically identify both shared and unique drivers of podocyte injury in vitro. Finally, we demonstrate how targeting mechanosensitive pathways may lead to the development of novel therapeutics that protect podocytes in situ, in addition to the identification of early-disease onset biomarkers. We anticipate that these findings may hold great potential for developing effective clinical interventions and therapies for patients suffering from CKD.

Funding Source: Morgan A. Burt is supported by an NSF Graduate Research Fellowship, awarded 2018
Keywords: hiPSC-derived podocytes, chronic kidney disease, in vitro disease models

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EXPLORING THE USE OF PROANGIOGENIC POROUS TEMPLATED HYDROGEL SCAFFOLDS TO IMPROVE NEPHRON PROGENITOR CELL ENGRAFTMENT BENEATH THE KIDNEY CAPSULE

Vincent, Thomas A. - *Bioengineering, Institute for Stem Cell & Regenerative Medicine at the University of Washington, Seattle, WA, USA*
Blackburn, Sophie - *Bioengineering, University of Washington, Seattle, WA, USA*
Chen, Ningjing - *Bioengineering, University of Washington, Seattle, WA, USA*
Ratner, Buddy - *Bioengineering, University of Washington, Seattle, WA, USA*
Freedman, Benjamin - *Medicine, University of Washington, Seattle, WA, USA*

Abstract: Adult human kidneys are comprised of approximately one million filtration units called nephrons that finish developing during gestation. If the kidney is acutely injured or damaged by an underlying chronic condition, the nephrons are unable to sufficiently repair themselves and progressively deteriorate. Treatments for end stage kidney failure are transplantation or dialysis which both have major disadvantages. The creation of stem cell derived kidney organoids has raised the possibility of achieving an alternative regenerative medicine-based therapeutic to restore renal function. However, studies in vivo have not yet achieved clinically significant integration of implanted cells with the host kidney. This research centers upon the development of new strategies to promote functional tissue engraftment through the use of proangiogenic uniformly porous templated hydrogel scaffolds alongside implanted stem cell-derived nephron progenitor cells (NPCs). Our research shows that implantation of NPCs beneath the kidney capsule of NOD-SCID mice leads to the development of renal structures that include podocytes, and proximal and distal tubules. The podocytes are able to interact with host vasculature to form chimeric glomerular structures. These structures appear more robust in comparison to implanted differentiated kidney organoids. Parallel experiments also show that the implantation of acellular uniform porous templated scaffolds beneath the kidney capsule allows for high cell infiltration from the surrounding tissue including vascularization without the use of cytokines such as VEGF. They also elicit less of a fibrotic encapsulation response, compared to non-porous implants of the same material. Preliminary results from in vitro scaffold cellularization experiments have shown that organoids can be differentiated on the surface of these porous scaffolds within a 3D extracellular matrix. Together, these experiments have validated the initial proof-of-principle for combining these two technologies. Next steps will be to optimize cell-seeding methodology and implant NPC-seeded scaffolds beneath the kidney capsule. This research is unveiling ways to utilize the unique properties of both NPCs and hydrogel scaffolds in synergistic ways that improve overall integration of regenerated tissue.

Funding Source: NIDDK, ReBuilding a Kidney Foundation, U.S.-Israel Binational Science Foundation, Institute for Stem Cell and Regenerative Medicine Pilot Award
Keywords: Kidney, Regeneration, Scaffold

TOPIC: MUSCULOSKELETAL

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NOVEL HIGH-THROUGHPUT OPTOGENETICS SYSTEM TO ASSESS FUNCTIONAL HUMAN NEUROMUSCULAR JUNCTION PROPERTIES

Miranda, Helen C. - *Genetics and Genome Sciences, Case Western Reserve University, Cleveland Heights, OH, USA*
Chen, Daniel - *Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA*
Philippidou, Polyxeni - *Neurosciences, Case Western Reserve University, Cleveland, OH, USA*

Abstract: Neuromuscular junctions (NMJs) are specific synapses that connect motor neurons to skeletal muscle and therefore are primarily responsible for voluntary movement. The degeneration

of this system can lead to symptoms that are observed in motor neuron and neuromuscular diseases. There is a substantial amount of studies on the cellular and molecular composition of NMJs in animal models, such as mouse and fly, whilst the knowledge on human NMJs is scarce due to obvious ethical concerns and relative inaccessibility of samples. In this work, we use human induced pluripotent stem cells (iPSCs) to generate motor neurons and skeletal muscles and co-culture these two cell types to form an in vitro humanized NMJ system. IPSC-derived NMJs were characterized morphologically by a-bungarotoxin staining. We also observed that when iPSC-derived skeletal muscles and motor neurons were co-cultured in the presence of agrin, there was a significant increase in the expression of acetylcholine receptor (AChR). Furthermore, we performed functional analysis of the iPSC-derived motor neuron and skeletal muscles separately by a multielectrode array (MEA) system using the Maestro instrument (Axion Biosystems). Both isolated cultures display spontaneous spike activity that increases over time, indicative of maturation of the cultures. The quantification of the spontaneous contractions of the iPSC-derived skeletal muscle cultures enabled the further optimization of the iPSC-derived NMJs functional analyses using the MEA system. In the iPSC-motor neuron and skeletal muscle co-culture, we observed increased spike frequency when compared to skeletal muscle alone. We have also taken advantage of optogenetics and transduced the iPSC-derived motor neurons with a lentivirus harboring the humanized channelrhodopsin (ChR2) under the control of the synapsin promoter. Transduced motor neurons co-cultured with skeletal muscle displayed an increase in number the of Bursts when subjected to light stimulation using the LUMUS apparatus (Axion Biosystems). To our knowledge, we are the first laboratory to optimize the MEA system for quantitation of iPSC-derived skeletal muscle activity and to measure functional human NMJs. We are currently using this system to investigate NMJ phenotypes associated with motor neuron disease pathogenesis.

Funding Source: R01NS121374 K01NS116119
Keywords: neuromuscular junctions, motor neuron disease, skeletal muscle

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FUNCTIONAL IMPACT OF ALTERNATIVE SPLICING ON THE TRANSCRIPTOMIC LANDSCAPE AND FATE OF MULTIPOTENT SKELETAL STEM CELLS

Butler, M. Gohazrua K. - *Surgery, Stanford University, Alamo, CA, USA*
Ambrosi, Thomas - *Surgery, Stanford University, Palo Alto, CA, USA*
Chan, Charles - *Surgery, Stanford University, Palo Alto, CA, USA*

Abstract: The essence of a cell's identity and function lies in its transcriptomic landscape, including the variety of protein isoforms that arise from alternative splicing (AS). Of all protein-coding genes in the human genome, greater than 90% undergo post-translational AS, giving rise to many unique isoforms from a single gene. Through rearrangement of functional domains prior to translation, AS allows a single gene to encode a variety of proteins that may function in varying degrees of similarity or differ entirely in their activity. Recent advances in our understanding of the human skeletal stem cell (hSSC) and its niche have demonstrated the need to examine AS as it relates to development, aging, loss of skeletal regenerative capacity and skewing of hSSCs towards non-skeletogenic lineage fates. Moreover, mounting evidence that the progression of osteosarcoma corresponds with aberrations in the AS machinery, producing cellular phenotypes that result in malignant tumors of the bone. In the present study, we aimed to characterize the relationship between AS and maintenance of hSSCs and their niche. We evaluated the expression profile of aged versus young SSC's using GEXC microarray data and found differences in the expression of critical AS machinery in young versus aged human and mouse SSCs. We developed an RNA-sequencing analytical pipeline to discover key RNA-binding proteins (RBPs) involved in alternative splicing of osteogenesis-related pathways and found two RBPs that are expressed during osteogenesis. Interference of these RBPs by siRNA reduced the capacity for hSSCs to differentiate into osteoblasts and prevented formation of bone. Similarly, inhibition of spliceosome complex SF3b prevented osteoblast differentiation in both young and aged hSSCs. Using osteosarcoma cell lines U2OS and SAOS2 we found that inhibition of SF3b also reduces resistance to methotrexate, a gold-standard chemotherapeutic. These findings reveal a promising lead to understanding the elements of skeletal aging and potential treatment for intractable cancers such as osteosarcoma.

Keywords: Skeletal stem cells, Alternative splicing, Osteosarcoma

TOPIC: NEURAL

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TROPISM OF SARS-COV-2 IN HUMAN CORTICAL ASTROCYTES

Mukhtar, Tanzila - *Neurology, University of California, San Francisco, CA, USA*
Andrews, Madeline - *Neurology, University of California, San Francisco, CA, USA*
Eze, Ugomma - *Neurology, UCSF, San Francisco, CA, USA*
Simoneau, Camille - *Gastroenterology, Gladstone Institutes, San Francisco, CA, USA*
Ross, Jayden - *Anatomy, UCSF, San Francisco, CA, USA*
Parikshak, Neelroop - *Neurology, UCSF, San Francisco, CA, USA*
Wang, Shaohui - *Neurology, UCSF, San Francisco, CA, USA*
Zhou, Li - *Neurology, UCSF, San Francisco, CA, USA*
Koontz, Mark - *Ophthalmology, UCSF, San Francisco, CA, USA*
Tabata, Takako - *Gastroenterology, UCSF, San Francisco, CA, USA*
Perez, Yonatan - *Neurology, UCSF, San Francisco, CA, USA*
Wang, Li - *Neurology, UCSF, San Francisco, CA, USA*
Mostajo-Radji, Mohammed - *Neurology, UCSF, San Francisco, CA, USA*
Velmeshev, Dmitry - *Neurology, UCSF, San Francisco, CA, USA*
Donohue, Kevin - *Anatomy, UCSF, San Francisco, CA, USA*
Shin, David - *Neurology, UCSF, San Francisco, CA, USA*
Salma, Jahan - *Neurology, AKU, Karachi, Karachi, Pakistan*
Pollen, Alex - *Neurology, UCSF, San Francisco, CA, USA*
Nowakowski, Tomasz - *Anatomy, UCSF, San Francisco, CA, USA*
Ullian, Erik - *Ophthalmology, UCSF, San Francisco, CA, USA*
Kumar, Renuka - *Gastroenterology, Gladstone Institutes, UCSF, San Francisco, CA, USA*
Winkler, Ethan - *Neurosurgery, UCSF, San Francisco, CA, USA*
Crouch, Elizabeth - *Neonatology, UCSF, San Francisco, CA, USA*
Ott, Melanie - *Gastroenterology, Gladstone Institutes, San Francisco, CA, USA*
Kriegstein, Arnold - *Neurology, UCSF, San Francisco, CA, USA*

Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) readily infects a variety of cell types impacting the function of vital organ systems, with particularly severe impact on respiratory function. Neurological symptoms, which range in severity, accompany as many as one third of COVID-19 cases, indicating a potential vulnerability of neural cell types. To assess whether human cortical cells can be directly infected by SARS-CoV-2, we utilized stem cell-derived cortical organoids as well as primary human cortical tissue, both from developmental and adult stages. We find significant and predominant infection in cortical astrocytes, in both primary tissue and organoid cultures, with minimal infection of other cortical populations. Infected and bystander astrocytes have a corresponding increase in inflammatory gene expression, reactivity characteristics, increased cytokine and growth factor signaling, and cellular stress. Although human cortical cells, and particularly astrocytes, have no observable ACE2 expression, we find high levels of coronavirus co-receptors in infected astrocytes, including CD147 and DPP4. Decreasing co-receptor abundance and activity reduces overall infection rate and increased expression is sufficient to promote infection. Thus we find potential ACE2-independent tropism of SARS-CoV-2 for human astrocytes, resulting in inflammatory gliosis-type injury.

Keywords: Viral tropism, astrocytes, inflammation

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THERAPEUTIC EFFECT OF NEURAL-INDUCED HUMAN ADIPOSE TISSUE-DERIVED STEM CELL-CONDITIONED MEDIUM DURING ROTENONE-INDUCED PARKINSON'S DISEASE IN SH-SY5Y CELLS

Ramalingam, Mahesh - *Department of Physiology, Chonnam National University Medical School, Hwasun-gun, Korea*
Hwang, Jinsu - *Department of Physiology, Chonnam National University Medical School, Hwasun, Korea*
Cho, Hyeong-Ho - *Department of Otolaryngology-Head and Neck Surgery, Chonnam National University Hospital and Medical Shool, Gwangju, Korea*
Kim, Byeong C. - *Department of Neurology, Chonnam National University Hospital and Medical Shool, Gwangju, Korea*
Kim, Eungpil - *Jeonnam Biopharmaceutical Research Center, Jeonnam Biopharmaceutical Research Center, Hwasun, Korea*
Jeong, Han-Seong - *Department of Physiology, Chonnam National University Medical School, Hwasun, Korea*
Jang, Sujeong - *Department of Physiology, Chonnam National University Medical School, Hwasun, Korea*

Abstract: Adult mesenchymal stem cells (MSCs) derived from the adipose tissue have been proven for treating human diseases. In addition, MSCs can be differentiated into functional neurons using basic fibroblast growth factor and forskolin. The beneficial effects of MSCs are mainly attributed to their secretomes mainly consist of numerous neurotropic factors, microRNA, proteasomes, and extracellular vesicles drawn much attention for treating neurodegenerative diseases. Rotenone (ROT), a naturally occurring piscicide, inhibits mitochondrial complex I, leading to reaction oxygen species formation, which causes neurodegeneration and alpha-synuclein (alpha-syn) aggregation and consequently Parkinson's disease. We had previously found that a neurogenic differentiated human adipose tissue-derived stem cell-conditioned medium (NI-hADSC-CM) was protective against ROT-induced alpha-syn toxicity in SH-SY5Y cells. In this present study, ROT for 48 h significantly decreased the phospho (p)-mTORC1/total (t)-mTOR, p-mTORC2/t-mTOR, and p-/t-ULK1 ratios and ATG13 level by increasing the DEPTOR level and p-/t-AMPK ratio. Moreover, ROT increased the p-/t-Akt ratio and glycogen synthase kinase-3eta (GSK3beta) activity by decreasing the p-/t-ERK1/2 ratios and beclin-1 level. ROT also promoted the lipidation of LC3B-I to LC3B-II by inducing autophagosome formation in Triton X-100-soluble and -insoluble cell lysate fractions. Additionally, the levels of ATG3, 5, 7, and 12 were decreased along with those of lysosomal LAMP1, LAMP2, and TFEB. However, NI-hADSC-CM treatment increased the p-mTORC1, p-mTORC2, p-ULK1, p-Akt, p-ERK1/2, ATG13, and beclin-1 levels and decreased the p-AMPK level and GSK3beta activity in response to ROT-induced toxicity. Additionally, NI-hADSC-CM restored the LC3B-I level, increased the p62 level, and normalized the ATG and lysosomal protein amounts to control levels. Autophagy array revealed the presence of the secreted autophagy related proteins in NI-hADSC-CM could be crucial in the neuroprotection. Taken together, our results showed that the effects of NI-hADSC-CM on the autophagy signaling pathways can alleviate the effects of Parkinson's disease.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF-2021R1I1A3060435, NRF-2020R1I1A3070388, and NRF-2020R1F1A1076616); and Jeollanam-do Science and Technology R&D Project, Korea.
Keywords: Parkinson's disease, autophagy, mesenchymal stem cells

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SYSTEMATIC ASSESSMENT OF IPSC-DERIVED NEURONAL AND GLIAL CELLS FOR INVESTIGATING NEUROLOGICAL AND NEURODEGENERATIVE DISEASE RISK LOCI

Gallagher, Michael D. - *Whitehead Institute, Cambridge, MA, USA*
Aydin, Zeynep - *Whitehead Institute, Cambridge, MA, USA*
Bell, George - *Whitehead Institute, Cambridge, MA, USA*
Cheng, Yiran - *Whitehead Institute, Cambridge, MA, USA*
Corradin, Olivia - *Biology, Whitehead Institute/MIT, Cambridge, MA, USA*
Du, Wenjuan - *Biology, Whitehead Institute/MIT, Cambridge, MA, USA*
Hazel, Kathryn - *Whitehead Institute, Cambridge, MA, USA*
Jaenisch, Rudolf - *Biology, Whitehead Institute/MIT, Cambridge, MA, USA*

Abstract: Neurological and neurodegenerative diseases (NDs) are a diverse group of debilitating conditions that impose substantial burdens on quality of life, healthcare systems and world economies. Genetic risk is known to play a major role in the development of NDs, with estimated heritabilities ranging from ~40-80%, and consistently, genome-wide association studies (GWAS) have identified thousands of single nucleotide polymorphisms (SNPs) that correlate with risk for many NDs. However, translating these statistical associations into pathophysiological insights has been challenging due to the complexity and cell type-specificity underlying GWAS-identified risk loci. Therefore, we have performed a systematic assessment of the ability of male and female iPSC-derived neural progenitors, neurons, astrocytes and microglia to recapitulate in vivo risk SNP mechanisms for a wide range of NDs. First, we identified the active enhancers in these cell types and found that despite poor concordance between iPSC-derived and ex vivo primary neuronal cell enhancers, iPSC-derived astrocyte and microglial enhancers clustered more closely with those of their primary counterparts. Strikingly, the

enrichment of ND risk SNPs in cell type-specific enhancers was very similar between primary astrocytes and microglia and their iPSC-derived counterparts, suggesting that despite well documented differences between in vivo and in vitro glial cells, current iPSC-based models may be well suited to study the role of ND risk SNP mechanisms in glia. To complement these analyses, we analyzed RNA-seq and H3K27ac ChIP-seq data to identify ND risk loci exhibiting allelic imbalance in mRNA levels and/or promoter and enhancer activity in all four cell types, as such effects are hallmarks of risk SNP-mediated regulatory effects. We hypothesize that these loci influence ND risk through regulatory effects on one or more target genes, and we aim to systematically identify these target genes by performing high throughput epigenome editing followed by scRNA-seq (Perturb-seq). These efforts provide a high level view of the relevance of iPSC-derived neuronal and glial cells for studying ND risk loci, and will shed new light on specific genes that influence ND risk by altering neuronal and glial function.

Funding Source: National Institutes of Health Novo Nordisk
Keywords: neurological disease, neurodegenerative disease, iPSC

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SINGLE-CELL RNA-SEQ OF HPSC-DERIVED NEURAL CELL TYPES REVEALS EARLY GLIA SPECIFIC DIFFERENTIATION TRAJECTORIES

Jovanovic, Vukasin M. - *Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), Rockville, MD, USA*
Slamecka, Jaroslav - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Hirst, Marissa - *Bioinformatics, Rancho BioSciences, San Diego, CA, USA*
Galbraith, David - *Bioinformatics, Rancho BioSciences, San Diego, CA, USA*
Sen, Chaitali - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Tristan, Carlos - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Ormanoglu, Pinar - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Singeç, Ilyas - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*

Abstract: During brain development radial glia cells (RGCs) generate large numbers of neurons followed by giving rise to astrocytes, a process that is known as the “gliogenic switch” of neural lineage progression. While neurogenesis has been extensively characterized, the underlying molecular mechanisms whereby RGCs differentiate into astrocytes remain elusive. Here, we performed single-cell mRNA sequencing (scRNA-seq) of astrocytes derived from human pluripotent stem cells (hPSC) by using a new highly efficient approach that achieves direct RGC-to-astrocyte conversion in the absence of neurogenesis. The single-cell transcriptome of these directly differentiated astrocytes was then compared to commercially obtained astrocytes and cortical glutamatergic neurons (FUJIFILM CDI). Altogether, we profiled the transcriptomes of 12,771 cells and unbiased clustering identified 11 transcriptionally distinct cell clusters. After data integration with single-cell transcriptomes of the developing human cortex, as expected, cluster signatures corresponded to 3 broad categories: radial glia, astrocytes, and excitatory cortical neurons. An additional small cell population clustered close to a choroid plexus signature. Next, to better understand cell type-specific molecular identities, we performed slingshot analysis and identified three different trajectories mapping transition of RGCs into two branches representing astrocytes and one “teleporting” RGCs (in the absence of neurogenesis transitional states were not detected) to excitatory neurons. To relate gene expression variation to each of the cell types in pseudotime along the mapped trajectories, we performed trajectory-based differential expression analysis (trade-Seq) and identified a new gene module that may determine early glial fate choice of RGCs at the expense of neurogenesis.

Keywords: Radial Glia Cells (RGCs), Astrocytes, Single-cell RNA-seq

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SINGLE-CELL MEASUREMENT OF THE EFFECTS OF DIFFERENTIAL MTORC1 SIGNALING DURING BRAIN DEVELOPMENT

Chalkley, Mary-Bronwen - *Cell & Developmental Biology, Vanderbilt University, Nashville, TN, USA*
Geben, Laura - *Pharmacology, Vanderbilt University, Nashville, TN, USA*
Brockman, Asa - *Cell & Developmental Biology, Vanderbilt University, Nashville, TN, USA*
Ess, Kevin - *Neurology, Vanderbilt University Medical Center, Nashville, TN, USA*

Abstract: The mechanistic target of rapamycin complex 1 (mTORC1) is a key regulator of cell size and growth in healthy development. Mutations in this pathway result in “mTORopathies,” wherein patients suffer multiple neurological symptoms and have abnormal tissue patterning. mTOR is active in neural stem/progenitor cells (NSPCs), but relatively little is known about which downstream biochemical and cell biological events drive specific patient phenotypes. In mammals, NSPCs exist in spatially and temporally organized populations that are variably proliferative or quiescent. Our data showed that different postnatal NSPCs have differing levels of mTORC1 signaling, but the time when these signaling differences first emerge and their relationship to stem cell activation and lineage commitment is unknown. Imaging and flow cytometry studies in the mouse revealed that embryonic NSPCs mirror their postnatal counterparts and have distinct levels of mTORC1 signaling depending on their dorsal-ventral identity. However, the pattern of mTORC1 signaling in prenatal NSPCs following quiescence induction differs from postnatal cells. Downstream mTOR targets also respond differentially to quiescence induction and are modified independently, not coordinately, in embryonic cells. To examine phenotypes in an mTOR disease model, we used patient-derived induced pluripotent stem cells (iPSCs) with a heterozygous mutation in TSC2, as well as isogenic homozygous mutant and wild-type lines. Microscopy, immunoblotting, and mass cytometry revealed aberrant early neurodevelopment in mutant iPSCs. Homozygous mutant NSPCs exhibit altered behavior during in vitro differentiation, including changes in early multicellular structures and misexpression of key transcription factors associated with lineage commitment. As expected, mutant cells have elevated mTORC1 activity, and preliminary analyses indicate that this activation differs between dorsal and ventral populations. Collectively, these data suggest that mutations in the mTORC1 pathway have early effects on proper neural development that are position- and stage-specific. Understanding the developmental requirements for mTORC1 signaling will be

essential to tailoring treatment and determining whether prenatal treatment for mTORopathies should be pursued.

Keywords: Neural Stem Cell, Tuberous Sclerosis Complex, Organoid

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ROLE OF DISTAL REGULATORY ELEMENT IN MAMMALIAN NEURAL DEVELOPMENT

Banno, Satoe - *Physiology, Keio University School of Medicine, Shinjuku-ku, Japan*
Mizota, Noriko - *Physiology, Keio University School of Medicine, Shinjuku-ku, Japan*
Sanosaka, Tsukasa - *Physiology, Keio University School of Medicine, Shinjuku-ku, Japan*
Kohyama, Jun - *Physiology, Keio University School of Medicine, Shinjuku-ku, Japan*
Okano, Hideyuki - *Physiology, Keio University School of Medicine, Shinjuku-ku, Japan*

Abstract: During central nervous development (CNS), cell-type specific gene expressions are regulated in precise space and time. Such a transcription is determined by regulatory elements including tissue-specific promoters and enhancers. Recently, it has become clear that enhancer RNAs (eRNAs) are expressed from enhancer regions and functions in the regulation of gene transcription. Sox2 is one of the transcriptional regulators, which is involved in the maintenance of neural progenitors, and its expression is believed to be regulated by distinct distal enhancers during CNS development. Here, we focused on one of distal enhancer region of Sox2 (Sox2-de1), which displayed unique activity during CNS development. We found expression of eRNAs corresponding to the regions of Sox2-de1 in E14 mouse brain. To further gain insight into importance of the Sox2-de1, the mice of Sox2-de1-homozygous deletions were generated using CRISPR-Cas9 genome editing technology. Although the deletion mice are viable and did not have a pronounced difference in appearance, we eventually found the formation of heterotopias in the E18 cortex, indicating the importance of Sox2-de1 in precise formation of cortical structure.

Keywords: enhancer RNA, heterotopias, Sox2

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REGULATION OF NEURAL STEM CELL DIFFERENTIATION AND BRAIN DEVELOPMENT BY MGAT5-MEDIATED N-GLYCOSYLATION

Flanagan, Lisa - *Neurology, University of California, Irvine, CA, USA*
Yale, Andrew - *Neurology, University of California, Irvine, CA, USA*
Kim, Estelle - *Neurology, University of California, Irvine, CA, USA*
Nourse, Jamison - *Neurology, University of California, Irvine, CA, USA*
Salvatus, Marc - *Neurology, University of California, Irvine, CA, USA*
Hunt, Robert - *Anatomy and Neurobiology, University of California, Irvine, CA, USA*
Monuki, Edwin - *Pathology and Laboratory Medicine, University of California, Irvine, CA, USA*

Abstract: Cell differentiation is a complex process critical for proper organ development and function. In the brain, undifferentiated neural stem and progenitor cells (NSPCs) encounter extracellular signals that bind plasma membrane proteins and impact differentiation. Plasma membrane proteins are regulated by N-linked glycosylation, making it possible that glycosylation plays a critical role in cell differentiation. However, links between specific glycosylation enzymes and NSPC differentiation have not been described. We assessed enzymes that control N-glycosylation in NSPCs and found loss of the enzyme responsible for generating the most highly-branched N-glycans, Nacetylglucosaminyltransferase V (MGAT5), led to specific changes in NSPC differentiation in vitro and in vivo. Mgat5 homozygous null NSPCs in culture formed more neurons and fewer astrocytes compared to wild-type controls, showing that glycosylation impacts stem cell fate. In the brain cerebral cortex, loss of MGAT5 accelerated neuronal differentiation. Rapid neuronal differentiation caused depletion of progenitors in the NSPC niche, resulting in a decrease in the numbers of neurons formed in Mgat5 null mice. Thus, the glycosylation enzyme MGAT5 plays a critical and previously unrecognized role in cell differentiation and early brain development.

Funding Source: NSF IOS-2019400 (LAF), NSF CAREER Award IOS-1254060 (LAF), NIH NINDS T32 NS082174 (predoctoral fellowship to AY), Brython Davis Fellowship (AY), NIH NCRR and NCATS through Grant UL1 TR001414 (Pilot Grant to LAF)
Keywords: neuron, astrocyte, cerebral cortex

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MODELING SYNAPTIC PLASTICITY ON HIPSC-DERIVED NEURONAL NETWORKS USING MULTI-ELECTRODE ARRAYS (MEAS)

Pre, Deborah - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Wooten, Alexander - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Neil, Ashley - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Zhou, Haowen - *CPCCG, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Bang, Anne - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*

Abstract: Synaptic plasticity is a crucial aspect of neuronal function. Long-term potentiation (LTP), a form of Hebbian plasticity that leads to a long-lasting strengthening of synapses in response to specific types of stimulation using a positive feedback process, has been extensively investigated. More recently, homeostatic plasticity (HP) has been described as a negative feedback process that plays a fundamental role in adjusting synaptic strength to offset excessive excitation or inhibition. It is unclear how LTP and HP, which share common downstream mechanisms, are integrated in the same network, both in physiological and disease contexts. Establishing approaches to investigate these forms of synaptic plasticity and their interplay in human models is an essential step towards developing relevant disease models. Thus, we designed MEA-based assays to model LTP and HP on human iPSC-derived neuronal networks. We show that chemical induction of LTP (cLTP) via cAMP elevation, potentiates network activity, increasing both firing rate and network burst frequency, for up to 72 hours after drug washout. We demonstrate that these effects are independent of the NMDAR and partially dependent

on BDNF and other unidentified factors released into the medium. We also observed cLTP-induced neuronal activity-regulated gene expression and a transcriptomic signature with both canonical and unique features. In parallel, we developed models of synaptic scaling, a form of HP, where we show that chemical induction that chronically increases or decreases activity induces compensatory mechanisms and a return to baseline activity within 24 hours. We observe that chronic AMPAR blockage with CNQX induces a synaptic-upscaling-like response that is NMDAR-dependent, and partially mediated by BDNF. In contrast, chronic exposure to the epileptogenic drug 4-AP, induces synaptic downscaling that is REST-mediated. In summary, the assays we have developed offer the opportunity to investigate different forms of plasticity on hiPSC neuronal networks, a powerful approach in the context of human genetics reflected by hiPSC models of neurological disease. Moreover, the higher throughput 48-well MEA format enables use of these assays for drug screening, optimization of culture conditions, and phenotype discovery across panels of patient lines.

Funding Source: This work was supported by grants from United States Department of Defense, US Air Force (FA8650-18-2-6971 to A.G.B.), National Institutes of Health (U19MH106434 to A.G.B.), and the Viterbi Family Foundation
Keywords: Synaptic plasticity, human induced pluripotent stem cells, Multi-Electrode Arrays

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METFORMIN MEDIATED FUNCTIONAL RECOVERY OCCURS IN THE ABSENCE OF GFAP EXPRESSING, NEURAL STEM CELL ACTIVATION FOLLOWING NEONATAL STROKE

Adams, Kelsey V. - *Institute of Medical Science, University of Toronto, ON, Canada*
Bollapragada, Adhirath - *Anatomy, University of Toronto, ON, Canada*
Bourget, Clara - *Institute of Medical Science, University of Toronto, ON, Canada*
Morshead, Cindi - *Anatomy, University of Toronto, ON, Canada*

Abstract: Neural stem cells and their progeny (neural precursor cells; NPCs) are promising candidates to promote neural repair. Previously, we demonstrated that administration of the anti-diabetes drug, metformin leads to motor and cognitive functional recovery in models of brain injury, including neonatal stroke. The metformin-mediated recovery was correlated with the activation of subependyma-derived NPCs including proliferation, migration to sites of injury and neural differentiation. We asked whether NPC activation was necessary and/or sufficient for the observed recovery using a conditional knock-out model of GFAP expressing neural stem cells and their progeny. Transgenic mice expressing thymidine kinase (TK) from the GFAP promoter (GFAP-TK) were used to selectively ablate proliferating GFAP-expressing neural stem cells in vivo. The ablation paradigm was performed in the early postnatal period and resulted in a significant, sustained depletion of neural stem cells for at least 2 months (the longest time examined) as revealed by the in vitro neurosphere assay and immunohistochemistry in brain sections. Importantly, ablation alone did not lead to functional deficits. As predicted, neonatal stroke resulted in significant impairments in motor and cognitive function which could be rescued by 1 week of metformin treatment. Most striking, the metformin-mediated recovery was also observed in neural stem cell-depleted mice. We found that functional recovery was correlated with increased neuroblast proliferation in the subependymal zone (SE), suggesting that an earlier cell, upstream of the GFAP+ neural stem cell, is able to repopulate the SE. Together, these findings provide insight into the cell-based mechanism underlying the metformin-mediated recovery post-HI.

Funding Source: This research was funded by the Network of Centres of Excellence, Ontario Brain Institute, Ontario Graduate Scholarship, the Carlton and Marguerite Smith Medical Research Fellowship and the University of Toronto Fellowship.
Keywords: Neural stem cell ablation, Metformin, Neonatal stroke

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INVESTIGATING THE PROTECTIVE MECHANISMS OF APOLIPOPROTEIN E2 (APOE2) IN MODULATING AMYLOID PRECURSOR PROTEIN (APP) PROCESSING

Srinivasan, Gayathri - *School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*
Frisch, Carlye - *School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*
Raman, Sreedevi - *School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*
Brookhouser, Nicholas - *Graduate Program in Clinical Translational Sciences, University of Arizona College of Medicine, Phoenix, AZ, USA*
Brafman, David - *School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*

Abstract: Apolipoprotein E (APOE) has been identified as a prominent risk factor for Alzheimer's disease (AD) with the E4 allele of APOE increasing the risk of AD 15-fold compared to APOE3. On the other hand, APOE2 has been suggested to decrease the likelihood of developing AD. However, the mechanisms by which APOE2 might exert such protective effects are unclear. This is specifically of interest, in the context of amyloid beta (Aβ) generation and clearance as an imbalance between the two processes has been hypothesised to drive AD pathogenesis. Here, we use a human induced pluripotent stem cell (hiPSC)-based model of AD to dissect the mechanisms by which APOE2 might modulate the processing of amyloid precursor protein (APP), which is proteolytically cleaved to form Aβ. We previously generated APOE2 isogenic lines from APOE3 familial AD patient-derived hiPSCs and differentiated them to neurons and astrocytes. Conversion of APOE3 to APOE2 resulted in preferential processing of APP in neurons by the non-amyloidogenic pathway that precludes Aβ generation. Lowered phosphorylation of APP, specifically at T668 in APOE2 neural cultures compared to APOE3 cultures suggest a role for differential APP phosphorylation in its processing. In addition, transcriptomic data revealed downregulation of key kinases that phosphorylate APP such as CDK5 and GSK3β in APOE2 cultures. To investigate the effect of APP phosphorylation on its processing, we inhibited CDK5 and GSK3β in our APOE isogenic neural cultures using small molecule inhibitors. Inhibiting these kinases had distinct effects on APP processing. While CDK5 inhibition lowered total and phosphorylated levels of APP as well as led to its preferential processing by the amyloidogenic pathway, GSK3β inhibition did not lower the levels of total or phospho-APP but altered the levels of amyloidogenic processing of APP. Overall, inhibiting these kinases altered APP processing not strictly by lowering its phosphorylation and further studies probing APP localization, α-, β- and γ-secretase levels and activity would provide insight into the mechanisms by which this might occur. In the fu-

ture, insights into these protective mechanisms of APOE2 would better inform therapeutics for AD.

Keywords: Alzheimer's disease, APOE, Kinases

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HUMAN CEREBRAL ORGANOID MODEL OF MATERNAL IMMUNE ACTIVATION REVEALS MICROCEPHALY PHENOTYPE WITH ACCELERATED NEURONAL MATURATION AT SINGLE CELL RESOLUTION

Imitola, Jaime - *Neurology, Genetics and Neuroscience, Deparment of Neurology University of Connecticut, Farmington, USA*
Hester, Mark - *Neuroscience, Nationwide Children's Hospital, Columbus, OH, USA*
Julian, Dominic - *Neuroscience, University of Arizona, Tuczon, AZ, USA*
Watanabe, Fumihiro - *Neuroscience, UConn Health, Farmington, CT, USA*
Hollingsworth, Ethan - *Genetics, UC Irvine, CA, USA*

Abstract: The pathological contributions of genetic risk factors to neurodevelopmental disorders, such as autism spectrum disorder and microcephaly, are becoming increasingly understood. By contrast, how environmental factors, like maternal infection and prenatal inflammation, impact human brain development and lead to neurodevelopmental disorders remain unclear. To study this mechanistic link, we adopted a single inflammatory perturbagen model on human brain development by exposing human cerebral organoids to the proinflammatory cytokine interferon-g (IFN-g). Release of IFN-g is an evolutionary conserved antiviral mechanism and is associated with maternal immune activation. Here, using single-cell RNA-sequencing (scRNA-seq), we unravel the developmental consequences of IFN-g exposure on early brain organogenesis at single-cell resolution. IFN-g exposure restricted organoid growth across several concentrations and timepoints by decreasing radial glia (RG) cell cycling, disrupting ventricular RG adherens junctions, and reducing outer RG proliferation. scRNA-seq revealed that IFN-g exposure induced premature neuronal differentiation of RG and cycling progenitors and accelerated neuronal maturation in a pan-neuronal fashion. Ectopic neuronal differentiation stems from cell-type-specific transcriptional rewiring by IFN-g, including upregulation of inflammatory and neurogenic genes. Transcriptomic dysregulation by IFN-g most closely reflects transcriptional changes seen in autism and maternal infections, opposed to genetic microcephaly or neuroinflammatory diseases. Altogether, our results highlight the feasibility of a single perturbation organoid paradigm to model environmental, non-genetic determinants of human brain development and reveal a novel molecular and cellular link between neuroinflammation involving a highly conserved interferon signature and ASD-like phenotypes.

Funding Source: UConn Health, NIH
Keywords: organoids, microcephaly, immunity

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GENOME-WIDE LOSS-OF-FUNCTION SCREENS REVEAL ESSENTIAL ROLES FOR HOX GENES DURING NEURONAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Yilmaz, Atilgan - *Azrieli Center for Stem Cells and Genetic Research, The Hebrew University, Jerusalem, Israel*
Bialer-Tsypin, Anna - *The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel*
Suhler, Roni - *The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel*
Benvenisty, Nissim - *The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University of Jerusalem, Israel*

Abstract: Essential gene networks for the maintenance of pluripotency and early germ layer differentiation have been recently identified by us by high-throughput functional genomics studies utilizing CRISPR/Cas9 technology. To this end, we generated a loss-of-function library in haploid human embryonic stem cells (hESCs) by targeting 18,000 coding genes with over 180,000 sgRNAs. Using this library, we identified essential genes for the normal growth and survival of hESCs and their differentiation into the ectoderm, mesoderm and endoderm. Due to the abundance of neurodevelopmental disorders, we recently focused on the more challenging task of mapping essential genes for more advanced stages of neuronal differentiation by utilizing our screening platform. By analyzing genes essential for neuronal differentiation for their association with brain disorders, we could demonstrate that the genes causing neurological conditions such as lissencephaly and colpocephaly have early differentiation phenotypes. Furthermore, our data allowed us to identify essential signaling pathways and transcription factors for neuronal differentiation in human. Essential transcription factors that we identified were enriched with a group of HOX genes showing stage-specific expression patterns. To understand their role during these differentiation events, we individually mutated the highest-ranking essential HOX gene, HOXA6, and its essential paralog, HOXB6, and performed gene expression analyses upon differentiation of these mutants. These analyses together with our genome-wide screen suggested that these two essential HOX factors have non-redundant functions during neuronal differentiation, while working synergistically to regulate the expression of neuron-specific signaling pathways. Interestingly, we also demonstrated that HOXA6 positively regulates the expression of the neighboring stage-specific HOXA cluster genes, while negatively regulating more posterior homologs. Overall, our work couples a genome-wide screening platform with a culture model of human neuronal differentiation and identifies essential genes for these cell state transitions.

Keywords: Neuronal differentiation, Human embryonic stem cells, Genome-wide CRISPR screens

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ELECTROPHYSIOLOGICAL PHENOTYPE CHARACTERIZATION OF HEALTHY AND DISEASED HUMAN IPSC-DERIVED MOTOR NEURONS BY MEANS OF HIGH-DENSITY MICROELECTRODE ARRAYS

Obien, Marie - *MaxWell Biosystems, Basel, Switzerland*
Buccino, Alessio - *D-BSSE, ETH Zurich, Basel, Switzerland*
Prack, Gustavo - *D-BSSE, ETH Zurich, Basel, Switzerland*

Kumar, Sreedhar - *D-BSSE, ETH Zurich, Basel, Switzerland*
Schröter, Manuel - *D-BSSE, ETH Zurich, Basel, Switzerland*
Jäckel, David - *MaxWell Biosystems, Zurich, Switzerland*
Frey, Urs - *MaxWell Biosystems, Zurich, Switzerland*
Fiscella, Michele - *MaxWell Biosystems, Zurich, Switzerland*
Hierlemann, Andreas - *D-BSSE, ETH Zurich, Basel, Switzerland*

Abstract: Induced pluripotent stem cell (iPSC) technology has increasingly made it easier to access human neurons for in vitro studies. Additionally, the study of neurodegenerative diseases benefited from the combination of iPSC and gene editing technologies: the diseases can now be modeled with human neurons, and the mechanisms behind these pathologies can be analyzed. In parallel, high-density microelectrode arrays (HD-MEAs) have become more widely used to non-invasively record extracellular activity of iPSC-derived neurons in vitro over weeks at unprecedented spatiotemporal resolution. In this work, we combined both HD-MEA and iPSC technologies to study the functional phenotype and development of a human motor neuron line modelling amyotrophic lateral sclerosis and its respective isogenic healthy line. We used an HD-MEA platform featuring 26'400 electrodes to explore the network, single-cell and subcellular-resolution electrophysiology metrics, such as network-burst properties, neuronal firing rate, and axonal conduction velocity. The two iPSC lines showed significant differences in network characteristics such as network burst duration, frequency, and shape, and in neuronal firing rate. Additionally, we extracted axonal features of the motor neurons and quantified functional and morphological metrics in a label-free approach. In summary, this work demonstrates that the combination of iPSC and HD-MEA technologies allows to successfully characterize healthy and diseased motor neurons and to identify their phenotypical differences. The presented methodology can potentially be utilized as a screening platform in the early phases of drug discovery for neurodegenerative diseases, such as ALS.

Funding Source: European Community through the ERC Advanced Grant 694829 "neuroXscales" and the proof-of-concept Grant 875609 "HD-Neu-Screen", the Swiss Project CTI-No. 25933.2 PFLS-LS, and SNSF under contract 205320_188910/1.
Keywords: microelectrode arrays, iPSC, electrophysiology

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GENERATION OF A GLIA-MIDBRAIN NEURON CO-CULTURE SYSTEM DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Knock, Erin - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
LeBlanc, Noemie - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Wang, Jinyuan - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Chan, Jeanne - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Eaves, Allen - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*

Abstract: Astrogliosis is a hallmark of Parkinson's disease, and astrocytes play key roles in neurodevelopment and neuroinflammation. To allow investigation of neuron-glia interactions in the context of disease modeling, we developed STEMdiff™ Midbrain Neuron and STEMdiff™ Astrocyte Differentiation and Maturation Kits to efficiently generate functional hPSC-derived midbrain neurons and astrocytes. hPSC-derived neural progenitor cells (NPCs) generated using the STEMdiff™ Neural Induction kit monolayer protocol were either plated into STEMdiff™ Astrocyte Differentiation Medium or STEMdiff™ Midbrain Neuron Differentiation Medium. Astrocytes were passaged every week using the recommended protocol. On the third passage, the medium was replaced with STEMdiff™ Astrocyte Maturation Medium and maintained for an additional two weeks. Midbrain neurons were passaged after one week into STEMdiff™ Midbrain Maturation Medium and cultured for two additional weeks. Cell identity was confirmed using immunocytochemistry for S100β, GFAP, and DCX (astrocytes), or BIIITUB and TH (midbrain neurons). We then co-cultured the astrocytes and neurons for one week. STEMdiff™ Astrocyte Differentiation and Maturation Kits resulted in a highly pure population of astrocytes (S100β: 72.0% ± 20.4%; GFAP: 33.8% ± 30.0%; DCX: 0.8% ± 1.5%; n = 6). STEMdiff™ Midbrain Neuron Differentiation and Maturation Kits produce a subpopulation of BIIITUB+ neurons expressing tyrosine hydroxylase (TH), the enzyme of dopamine biosynthesis (BIIITUB: 93.46% ± 6.93; TH: 16.57% ± 10.67; n = 13). Multielectrode array data show electrical activity up to 6 months in culture (up to 0.85 ± 0.33 Hz mean firing rate). Interestingly, the number of BIIITUB- and TH-positive neurons was higher in the astrocyte co-culture group than in the neuron-only control (BIIITUB: 2.76 ± 1.50-fold higher, P = 0.0003; TH: 2.63 ± 0.99-fold higher, P=0.01; n = 6, unpaired t-test), which indicates co-culture with astrocytes could lead to better survival of neurons in vitro. These data show that STEMdiff™ Midbrain Neuron Differentiation and Maturation Kits can generate functional hPSC-derived dopaminergic-midbrain neurons, which, used alongside STEMdiff™ Astrocyte Differentiation and Maturation Kits, create co-culture models suited for in vitro studies of neurodegeneration.

Funding Source: STEMCELL Technologies is a private for profit biotechnology company
Keywords: dopaminergic neuron, astrocytes, Parkinson's Disease

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FUNCTIONAL CHARACTERIZATION OF STEM CELL-DERIVED DOPAMINE NEURONS FOR THE TREATMENT OF PARKINSON'S DISEASE.

Tam, Edmund - *Translational Neuroscience, BlueRock Therapeutics, Long Island City, NY, USA*
Clark, Haley - *Translational Neuroscience, BlueRock Therapeutics, New York, NY, USA*
Ebel, Mark - *Discovery Analytics, BlueRock Therapeutics, New York, NY, USA*
Pereira, Elizabeth - *Computational Core, BlueRock Therapeutics, New York, NY, USA*
Purohit, Prasad - *Translational Neuroscience, BlueRock Therapeutics, New York, NY, USA*
Wilkinson, Dan - *Computational Core, BlueRock Therapeutics, New York, NY, USA*
Srinivas, Maya - *Discovery Analytics, BlueRock Therapeutics, New York, NY, USA*
Smith, Ryan - *Translational Neuroscience, BlueRock Therapeutics, New York, NY, USA*
Hudzik, Tom - *Translational Neuroscience, BlueRock Therapeutics, New York, NY, USA*
Petko, Alyssa - *Translational Neuroscience, BlueRock Therapeutics, New York, NY, USA*

Abstract: Human pluripotent stem cell-derived dopamine (DA) neuron progenitors show great promise for the applications in regenerative medicine, including authentic cell replacement for the treatment of Parkinson's disease. DA neurons generated from pluripotent stem cells are currently being tested for safety and tolerability in clinical trials (https://clinicaltrials.gov/ct2/show/study/NCT04802733). Here, we characterized a research-grade human stem cell-derived DA neuron cell therapy product, DA01, applying molecular and functional approaches over the course of in vitro neuronal cell maturation. Using a multi-electrode array (MEA) platform to measure population activity, we found that DA01 cells reach a mean firing rate of 2-8 Hz, typical of midbrain DA neurons, after extended maturation. Using the patch clamp technique to analyze activity from single cells, DA01 cells begin to show spontaneous spiking activity early in maturation, while IH currents are observed later in maturation. We used additional, complementary approaches to further characterize DA01 in vitro, profiling transcriptomic changes, ionic composition, and activity-dependent calcium signaling, as well as the release of DA. Importantly, DA01 cells showing significant in vitro functional activity express the gene encoding tyrosine hydroxylase (TH), in addition to other genes associated with midbrain DA neuron specification and function. In order to test the integrity of DA receptor function in our cell product, we found that quinpirole, a D2 receptor agonist, decreases population activity as expected. Finally, we extended our analysis to multiple independently produced lots of research-grade DA01 cell product, in order to understand batch-to-batch reproducibility of these characteristics. Taken together, these data confirm that DA01 neurons molecularly and functionally resemble canonical DA neurons over the course of their maturation.

Keywords: Dopamine, Electrophysiology, Parkinson's Disease

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EMERGENCE OF SYNAPTIC MODULES DURING EARLY HUMAN CORTICAL DEVELOPMENT

Zhou, Li - *RMB, University of California, San Francisco, CA, USA*
Wang, Li - *RMB, University of California, San Francisco, CA, USA*
Bhaduri, Aparna - *Department of Biological Chemistry, UCLA, San Francisco, CA, USA*
Bi, Qiuli - *RMB, University of California, San Francisco, CA, USA*
Wang, Shaohui - *RMB, University of California, San Francisco, CA, USA*
Moore, Anna - *Department of Biology, Temple University, Philadelphia, PA, USA*
Panagiotakos, Georgia - *RMB, University of California, San Francisco, CA, USA*
Alvarez-Buylla, Arturo - *RMB, University of California, San Francisco, CA, USA*
Kriegstein, Arnold - *RMB, University of California, San Francisco, CA, USA*

Abstract: Modeling human cortical development in vitro now urges more information about the early human cortical circuits, as normal brain development requires electrical activity triggered by long-range inputs or local circuits, but little is known about the emergence of local cortical circuits during human brain development. We explore physiological features and early intracortical connectivity in the developing human cortex during the second trimester, at the beginning of synaptogenesis. Single-cell Patch-seq demonstrates that electrophysiologically immature and morphologically simple cells in the cortical plate and marginal zone express genes for synaptic components. Ex vivo rabies tracing reveals the emergence of local synaptic modules in the cortical plate and subplate composed of immature excitatory neurons including migrating cells. Activity-dependent and independent spontaneous patterns of calcium activity can be observed in the synaptic modules. Furthermore, serotonin signaling regulates the formation of local synaptic modules through the activation of 5-HT2A receptors. Understanding the development of early intracortical circuits may shed light on the etiology of neurodevelopmental disorders and can inform in vitro models of human brain development.

Keywords: human cortical development, circuits, serotonin

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DYSREGULATION OF CYTOKINESIS BY MUTANT NS1BP UNDERLIES PROGERIA SYMPTOMS IN A NEWLY IDENTIFIED DISEASE

Yuan, Fang - *Neuroscience and Behavioural Disorders, Duke-NUS Medical School, Singapore, Singapore*
Reversade, Bruno - *Laboratory of Human Genetics & Therapeutics, Genome Institute of Singapore, A*STAR, Singapore, Singapore*
Zhang, Su-Chun - *Neuroscience and Behavioural Disorders, Duke-NUS Medical School, Singapore, Singapore*

Abstract: Aging is associated with cellular senescence characterized by growth arrest, DNA damage and senescence-associated secretory phenotypes. Mutations in lamin A (progerin) disrupts cell division and DNA damage, accelerating cellular senescence and resulting in Hutchinson-Gilford Progeria Syndrome (HGPS). We have encountered an undiagnosed family whose teenagers display progeria and severe neurological symptoms, including dyschromatosis, gray hairs, progressive motor deficits, and intellectual developmental delay. Whole genome sequencing revealed a homozygous mutation in the NS1BP gene. As an influenza virus nonstructural protein-1 binding protein, NS1BP functions as a modifier of the aryl hydrocarbon receptor pathway, as well as involvement in pre-mRNA splicing and F-actin organization. Structurally, NS1BP is a kelch protein and belongs to the family of adaptor proteins of the E3 ligase. Unlike other KLHL proteins, NS1BP does not appear to bind Culin3 directly; instead, it may regulate the proteasome system through interacting with KLHL20. The association between NS1BP and aging or neurological deficits has never been reported.To identify the processes that underlie the effect of mutant NS1BP on cellular senescence, we generated iPSCs from the patients' fibroblasts and corrected the mutation by Crispr/Cas9 before the isogenic iPSCs were differentiated to neural progenitors. We found that the mutant fibroblasts, iPSCs, and neural progenitors grew significantly more slowly with increased cell death. Cell cycle analysis revealed prolonged cell cycle and disrupted mitosis, corresponding to the observed aneuploidy and DNA damage. Mass Spectrum analysis of proteins, pulled down by NS1BP, revealed alteration in cytoskeleton proteins, especially actin and actin binding proteins. Indeed, actin dynamics and its polymerization/depolymerization was altered during mitosis of the mutant cell. We propose that mutant NS1BP dysregulates actin filament dynamics during cytokinesis, resulting in DNA damage and cellular senescence, a potential mechanism underlying the progeria symptoms of the patients.

Keywords: Progeria, Cytokinesis, Actin dynamics

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DIRECT CONVERSION OF SOMATIC FIBROBLASTS TO GLIAL CELLS TO MODEL AGE AND ALZHEIMER'S DISEASE

Goodman, Ryan - *Biology, San Diego State University, Salk Institute for Biological Studies, San Diego, CA, USA*
Jones, Jeffrey - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Neymeyer, Traci - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Revanna, Jasmin - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Kang, Austin - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Heard, Kelly - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Karanam, Ananth - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Hui, Lauren - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*
Gage, Fred - *Genetics, Salk Institute for Biological Studies, La Jolla, CA, USA*

Abstract: Alzheimer's disease (AD) is the most common type of dementia, accounting for 6.2 million cases worldwide. It is projected that by 2050, this number will double (12.7 million). The greatest known risk factor is advanced age, with the vast majority of those diagnosed being over the age of 65 years old. While neurons have been the main focus for investigations of AD, about half of the cells in the brain are glial cells, the majority of which are astrocytes. AD pathogenesis is complex and increasing evidence suggests non-cell autonomous contributions. While induced pluripotent stem cells (iPSCs) provide a powerful tool for modeling human disease, these cells reflect an embryonic state, and therefore do not maintain aspects of age. Our lab and others have applied direct conversion strategies that bypass normal development to directly convert aged somatic fibroblasts into neurons. To address contributions of aged glial cells to AD, a new direct conversion paradigm is required. Thus far, attempts to generate induced astrocytes from human fibroblasts have been challenging. We have developed a CRISPR-Cas9a mediated screen to identify factors sufficient for the direct conversion of fibroblasts into astrocytes. Using a guide-RNA library targeting promoters of every known transcription factor expressed in human astrocytes, we perturbed the fibroblast transcriptome at random and selected for cells that turn on the human astrocyte associated Glial Fibrillary Acidic Protein (GFAP) via a GFP reporter. We performed bulk and single cell RNA sequencing data and found enrichment of a subset of transcription factors associated with GFAP expression significantly altered the transcriptome of the cells. This new technique will allow for the study of aged and AD human astrocytes.

Funding Source: Salk Institute for Biological Studies, San Diego State University, California Institute for Regenerative Medicine
Keywords: Alzheimer's Disease, Direct Conversion, Astrocytes

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DETERMINATION OF MORPHOGEN PATTERNING EFFECTS AT A SINGLE LOCUS IN HUMAN MIDBRAIN DOPAMINERGIC CELL DEVELOPMENT

Ni, Anjie - *Department of Human Genetics, McGill University, Brossard, PQ, Canada*
Stumpf, Patrick Simon - *Institute for Computational Biomedicine, RWTH Aachen University, Aachen, Germany*
Peng, Huashan - *Department of Psychiatry, Douglas Hospital Research Institute, Montreal, QC, Canada*
Jefri, Malvin - *Department of Psychiatry, Douglas Hospital Research Institute, Montreal, QC, Canada*
Ernst, Carl - *Department of Human Genetics, McGill University, Montreal, QC, Canada*

Abstract: Cell patterning refers to cell identities that form in response to inductive signalling cues, which occurs via concentration gradients of morphogens. The timing, dose, and type of combinations of morphogens specify cell fate, but precisely how morphogens pattern developing cells is not well understood. To decipher temporal and dosage effects of combinations of morphogens, we used in vitro induction of stem cells to midbrain dopaminergic cells as a model system. We identified SULF1 as a critical cell patterning gene in midbrain dopaminergic progenitors which respond directly to midbrain inducers sonic hedgehog (SHH) and WNT. SULF1 protein is extracellular and it cleaves 6-O-sulfate groups from heparan sulfate to alter the extracellular matrix, which can influence morphogen binding to receptors. Our goal is to decipher the genomic effects of GLI and LEF binding to the SULF1 gene, where GLI and LEF are downstream transducers of SHH and WNT, respectively. With this system, we will understand how these morphogens act together in time and space to alter genomic structure and drive expression of SULF1 to influence midbrain cell fate. We suggest that WNT- and SHH-driven expression of SULF1 functions in a positive feedback loop to increase SHH and WNT morphogen activity outside the cell.

Keywords: morphogen signalling, cell patterning, human iPSC-derived midbrain NPCs

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DECIPHERING STRESS-RELATED SYMPATHETIC NEUROPATHOLOGY IN FAMILIAL DYSAUTONOMIA USING INDUCED PLURIPOTENT STEM CELL-BASED DISEASE MODELING

Wu, Hsueh Fu - *Biochemistry & Molecular Biology, University of Georgia, Athens, GA, USA*
Yu, Wenxin - *Animal & Dairy Science, University of Georgia, Athens, GA, USA*
Carey, Joseph - *Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA*
Lefcort, Frances - *Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA*
Liu, Hong-Xiang - *Animal and Dairy Science, University of Georgia, Athens, GA, USA*
Zeltner, Nadja - *Biochemistry & Molecular Biology, University of Georgia, Athens, GA, USA*

Abstract: Familial dysautonomia (FD) is a neurodevelopmental and neurodegenerative disorder that mainly affects the peripheral nervous system, especially the sympathetic nervous system (SNS). One of the most devastating symptoms in patients with FD

is the dysautonomic crisis, a severe dysregulation and dysfunction of the SNS triggered by stress. Interestingly, brain function which is the main stress responding center is minimally impacted in FD. This highlights the importance of the SNS in FD pathology. To date, the role of SNS dysfunction in FD, specifically, the pathological mechanism in sympathetic neurons (symNs) that leads to developmental defects, degeneration and FD crisis, remains unclear. A challenge of that is the lack of a accessible human model system comprised of symNs. Here, we adopt the human pluripotent stem cell (hPSC)-based disease modeling to investigate the defects in FD symNs. We show in early stages of differentiation, FD iPSCs display inefficient neural crest (NC) specification. When differentiating into sympathetic neuroblasts, FD NCs have a lower survival rate, yet remaining progenitor cells still generate symNs. These results reveal early developmental defects of SNS in FD. Using microelectrode array and calcium imaging, we found that FD symNs are spontaneously hyperactive. Accordingly, FD symNs drive higher cardiomyocyte beating in a co-culture system. We further confirmed symN hyperactivity using primary symN from mice lacking ELP1, the main gene mutation in FD. To understand the molecular cause of hyperactivity, we show that ELP1 rescue does not rescue hyperactivity, suggesting the complexity of FD mechanisms in human. In addition, we found decreased intracellular norepinephrine (NE) levels in FD symNs, suggesting impaired autoregulatory function. Indeed, FD symNs expressed less norepinephrine transporter (NET), a NE reuptake transporter, and displayed decreased NE reuptake ability. Treating control symNs with NET inhibitor increased their activity, which supports our hypothesis. Finally, we performed a mini drug screen and showed that our hPSC-based platform can be used for future FD drug discovery. Together, our studies show that symN hyperactivity caused by ill-controlled neurotransmitter release may contribute to FD pathology in patients.

Keywords: Familial dysautonomia, sympathetic nervous system, peripheral nervous system

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CHEMICALLY DEFINED SUBSTRATES IN A READY-TO-USE FORMAT THAT PROMOTE DIFFERENTIATION, ACCELERATED MATURATION AND NEURITE EXTENSION OF MULTIPLE NEURONAL SUBTYPES FOR SCREENING APPLICATIONS

Richards, William - *Stem Pharm, Inc., Madison, WI, USA*
Favreau, Peter - *Stem Pharm, Inc., Madison, WI, USA*
Erpelding, Owen - *Stem Pharm, Inc., Madison, WI, USA*
Parham, Kailyn - *Stem Pharm, Inc., Madison, WI, USA*
Lebakken, Connie - *Stem Pharm, Madison, WI, USA*

Abstract: Human neural cells manufactured from induced pluripotent stem cells (iPSCs) hold great promise for modeling neurodevelopmental disorders, screening for potential risks from environmental toxins, and use in regenerative cell therapies. However, most protocols require the use of complex, animal-derived substrates that need to be prepared prior to cell plating and introduce variability to culture outcomes. We have developed chemically defined substrates employing norbornene-functionalized polyethylene glycol (PEG) and synthetic peptides that can be applied to cell culture surfaces, dehydrated, packaged, sterilized, and stored, ready for re-hydration and use. We employed Design of Experiment (DOE) methodology utilizing Box-Behnken response surface modeling to screen for formulations that promoted cell viability, adhesion, and desired morphology. Our results indicate accelerated maturation of cortical glutamatergic and motor neurons as well as differentiation from neural precursor cells to glutamatergic neurons. These formulations were evaluated for compatibility with commercially available iPSC-derived neurons including GABAergic neurons and transcriptionally induced excitatory neurons. Transcriptional profiling using bulk RNASeq analysis was performed to compare neural cultures on the substrates with those cultured on standard substrates, including charged polymers (poly-lysines) or animal-derived substrates (PLO-Laminin or Geltrex). Cells cultured on Stem Pharm's hydrogel substrates demonstrated an increase in expression of gene ontology sets for neuronal maturation, neuron projection guidance, and cell-substrate adhesion in day seven samples; and enhanced cell differentiation, migration, signaling at the cell periphery and vesicles at day fourteen. The pre-plated substrates reduce time for experimental preparation, reduce the substrate complexity, eliminate ethical considerations of animal-derived substrates, and improve assay metrics when applied to screening campaigns.

Funding Source: NIH NINDS SBIR grants 1R43NS102088, and 2R44NS102088
Keywords: Drug Discovery, High Content Assay, Substrates

TOPIC: PANCREAS

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TRANSPLANTATION OF STEM CELL DERIVED BETA-LIKE CELLS TRIGGERS A SENESCENCE ASSOCIATED SECRETORY PHENOTYPE MARKED BY CD9

Shilleh, Ali H. - *Department of Pediatrics, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA*
Beard, Scott - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Anderson, Amanda - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Landry, Laurie - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Dwulet, JaeAnn - *Bioengineering and Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Benninger, Richard - *Bioengineering and Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Nakayama, Maki - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*
Russ, Holger - *Pediatrics, University of Colorado, Anschutz Medical Campus, Barbara Davis Center, Aurora, CO, USA*

Abstract: Cadaveric islet transplantation has been suggested as a cure for type 1 diabetes (T1D) but inadequate donor supply is a key challenge. We and others have recently developed a step wise differentiation protocol for large-scale production of stem cell derived insulin producing beta-like cells (sBC). sBCs can correct diabetes in preclinical animal models, demonstrating the promise of this stem cell-based approach. Recently we have shown that sBC in vitro are heterogenous with distinct subpopulations present. Similar, previous work using cadaveric human islet demonstrated the existence of 4 functionally distinct beta cell sub populations (β1 to 4) marked by surface molecules CD9 and/or ST8SIA1. In addition, a senescence associated secretory phenotype (SASP)-like beta cell subpopulation has been described,

that if ablated prevents the development of T1D in NOD mice. Using quantitative flow cytometry (FCM) analysis of sBC generated from multiple human pluripotent stem cell lines, we find a small but reproducible CD9+ sBC subpopulation in vitro. CD9+ sBC cells exhibit a SASP-like phenotype, as revealed by FCM and immune fluorescence staining (IF) of protein markers p21, IL6 and IL1B. scRNA-seq analysis of sBC further corroborated these findings. Human cadaveric beta cells revealed expression profiles suggestive of SASP in CD9+ labeled β2 and 4 subpopulations similar to sBC. GO analysis showed an enrichment in immune response genes in this population, suggesting that CD9+ human beta cells are more immunogenic and could play a potential role in autoimmune diabetes. Ca2+ imaging revealed that CD9+ sBC population respond to glucose challenge albeit significantly less so compared to CD9- sBC cells, suggesting partially impaired functionality. Engraftment of sBCs in immune deficient mice results in a ~12x fold increase in CD9+ sBC as detected by FCM and IF analysis. Similar to our in vitro findings, CD9+ sBC in vivo also exhibit a SASP phenotype. Preliminary data using an HLA matched beta cell and diabetogenic T cell co-culture system indicates increased immunogenicity of SASP beta cells. Overall, our study provides important insights for current cell therapy efforts, showing the prominent emergence of sBC with a SASP phenotype in vivo, a beta cell phenotype previously implicated in T1D diabetes onset and progression.

Keywords: direct differentiation, pancreatic beta cell, senescence associated secretory phenotype, Human pluripotent stem cells, cell therapy

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AUTOMATED PLATFORM TO DERIVE IPSC-PANCREATIC ORGANOIDS FOR POPULATION-SCALE MODELING OF TYPE 2 DIABETES

Cipriani, Filippo - *iPSC Models of Diabetes, The New York Stem Cell Foundation, New York, NY, USA*
Butt, Annie - *The New York Stem Cell Foundation, New York, NY, USA*
Dale, Nathan - *The New York Stem Cell Foundation, New York, NY, USA*
Tam, Edmund - *The New York Stem Cell Foundation, New York, NY, USA*
Zimmer, Matthew - *The New York Stem Cell Foundation, New York, NY, USA*
Goldberg, Jordan - *The New York Stem Cell Foundation, New York, NY, USA*
Paull, Daniel - *The New York Stem Cell Foundation, New York, NY, USA*
Bonnycastle, Lori - *National Human Genome Research Institute, Center for Precision Health Research, Bethesda, MD, USA*
Swift, Amy - *National Human Genome Research Institute, Center for Precision Health Research, Bethesda, MD, USA*
Narisu, Narisu - *National Human Genome Research Institute, Center for Precision Health Research, Bethesda, MD, USA*
Monsma, Frederick - *The New York Stem Cell Foundation, New York, NY, USA*
Solomon, Susan - *The New York Stem Cell Foundation, New York, NY, USA*
Erdos, Michael - *National Human Genome Research Institute, Center for Precision Health Research, Bethesda, MD, USA*
Collins, Francis - *National Human Genome Research Institute, Center for Precision Health Research, Bethesda, MD, USA*
Johannesson, Bjarki - *The New York Stem Cell Foundation, New York, NY, USA*
Noggle, Scott - *The New York Stem Cell Foundation, New York, NY, USA*

Abstract: Type 2 diabetes (T2D) is a genetically complex disease that requires large cohorts of patient-derived cells to experimentally establish defined model disease phenotypes. Although significant progress has been made towards large-scale production of pancreatic beta cells, current procedures for beta cell production use spinner flasks, which are not amenable to population-scale disease modeling. At NYSCF, we leveraged the NYSCF Global Stem Cell Array(R), our automated platform for iPSC derivation, to develop a fully automated, high-throughput platform for the directed differentiation of human pluripotent stem cells into pancreatic organoids comprising of functional beta cells and other hormonal cells. This approach improves the molecular properties of differentiated tissues; indeed, the automated platform yields better marker expression than the manual approach throughout the differentiation (PDX1+/NKX6+ at progenitor stage, ratio of INS+/GCG+ after maturation stage). Moreover, we closely monitored iPSC differentiation into pancreatic organoids via automated procedures for assessing 2D culture morphology and organoid dimension. We also implemented this differentiation pipeline with automated flow cytometry analysis to evaluate developmental marker expression profiling at every stage of the differentiation. Functional evaluations with Glucose-Stimulated Insulin Secretion (GSIS) assays showed consistent fold induction across a large cohort of iPSC-derived beta cells. Immunohistochemistry revealed a homogenous population of beta cells forming organoids. Our recent work shows that our automated platform for the derivation of functional pancreatic beta-cell organoids is optimized for detecting diverse phenotypes in population-scale disease modeling of diabetes. This sets the stage for cohort-scale, precision drug screening applications.

Keywords: Diabetes, Pancreatic Organoids, POPULATION-SCALE MODELING

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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COVID-19 NATURALLY EXPOSED PLACENTA-DERIVED MESENCHYMAL STEM CELLS PRESENT INCREASED MULTIPOTENTIALITY DURING PROLONGED IN-VITRO CELL CULTURE

Solis, Mairim A. - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama, Panama*
Fu, Cindy - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama, Panama*
Guerrero, Erika - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama, Panama*
Lopez-Verges, Sandra - *Department of Research in Virology and Biotechnology, Gorgas Memorial Institute for Health Studies, Panama, Panama*
Sanchez, Jaime - *Department of Obstetrics and Gynecology, Hospital Santo Tomas, Panama, Panama*
Vigil-De Gracia, Paulino - *Obstetrics and Gynecology, Complejo Hospitalario Dr. Arnulfo Arias Madrid, Panama, Panama*

Abstract: SARS-CoV-2, the virus responsible for COVID-19, is a highly contagious viral agent that has infected a high proportion of pregnant women worldwide. We have previously reported positive detection of SARS-CoV-2 in the placenta of COVID-19-positive pregnant women, with virus localized in chorionic villi endothelial cells. Although, detection of SARS-CoV-2 in the placenta does not seem to be a frequent event, it remained to be elucidated whether COVID-19 exerts any changes in the biological function of placenta-derived mesenchymal stem cells (PDMSCs). In this study, we characterized the multipotent capacity of PDMSCs derived from COVID-19-positive pregnant women (C-PDMSC) and to which degree these abilities are altered after several passages. C-PDMSC presented a wider cytoplasm and bigger cell size, similar to those observed in non-COVID-19-derived-PDMSC (NC-PDMSCs) cultured for 15 passages. The population doubling was higher in C-PDMSCs, reaching up to a 70% increase in cell proliferation compared to NC-PDMSCs. A twofold increase in the expression of CD105, CD90, and CD73 markers occurred in C-PDMSCs, whereas a boost in NANOG, OCT4 and SSEA4 was found only after long term culture of C-PDMSCs. Higher expression of the differentiation markers involved in chondrogenesis (ACAN, SOX9), adipogenesis (ALP, FEB4, CEPB), and osteogenesis (COL1, OPN, RUNX2, OCN) was present in C-PDMSC at early passages, however these expressions seemed to be almost lost afterwards. At advanced passages, a dramatic increase in the population of Beta-galactosidase positive cells was observed in NC-PDMSCs, consistent with a 20-times increase in the expression of the senescent marker, p21. However, no increase in senescence was found in C-PDMSCs, with levels of reactive oxygen species remained at least seven times lower than NC-PDMSCs. Together, these results suggest an increased multipotentiality in PDMSCs derived from COVID-19-positive patients. To our knowledge, this is the first study to underscore the biological impact of COVID-19 in mesenchymal stem cells derived from the placenta of SARS-CoV-2-positive pregnant women, which results will be beneficial for pregnancy monitoring and for the determination of their regenerative capability for future cell therapies.

Funding Source: This work was supported by Research Grants PFID-FID-2021-196 and the Sistema Nacional de Investigación from the Secretaria Nacional de Ciencia Tecnología e Innovación, L'Oreal for Women in Science, and the British Embassy of Panama.
Keywords: COVID-19, Plaenta-derived Mesenchymal stem Cells, Multipotency

TOPIC: PLURIPOTENT STEM CELLS

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SELF-ORGANIZED NODAL SIGNALING SYNERGIZES WITH WNT PATHWAY TO ENHANCE PRIMITIVE-STREAK FATES DIFFERENTIATION

Liu, Lizhong - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Nemashkalo, Anastasiia - *CINT/B11 Division, Los Alamos National Laboratory, Los Alamos, NM, USA*
Rezende, Luisa - *Department of Biosciences, Rice University, Houston, TX, USA*
Jung, Ji Yoon - *Department of BioSciences, Rice University, Houston, TX, USA*
Chhabra, Sapna - *Developmental Biology Unit, EMBL Heidelberg, Heidelberg, Germany*
Guerra, Cecilia - *Department of BioSciences, Rice University, Houston, TX, USA*
Heemserk, Idse - *Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Warmflash, Aryeh - *Department of BioSciences, Rice University, Houston, TX, USA*

Abstract: During gastrulation, the body plan of the early embryo is established as cells differentiate to the primary germ layers and the major body axes are specified. However, owing to the combined difficulties of measuring endogenous morphogen levels and observing development in utero, little is known how individual cells make fate decisions according to the morphogen signals that they perceive during gastrulation. Here we take advantage of a human gastruloid model to visualize endogenous Nodal protein in living cells, during the specification of germ layers. We show Nodal spreads through a transcriptional relay mechanism among adjacent cells. The timing of the spreading is controlled by Lefty and is important for the differentiation and positioning of mesodermal layer. We further dissect multiple points of synergy between Wnt and Nodal pathways by monitoring signal transducers and endogenous Nodal protein production in the presence of extracellular Wnt and Nodal ligands. We find the synergy between Wnt and Nodal pathways enhances differentiation of hESCs toward primitive streak fates. Overall, our study establishes a paradigm for self-organized tissue patterning by morphogen signals in a stem cell-based model.

Keywords: Gastrulation, Nodal and Wnt morphogen pathways, Self-organization

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MODELING CONTRACTILE DISEASES USING SCALABLE 3D ENGINEERED MUSCLE TISSUES FOR DRUG DISCOVERY

Luttrell, Shawn - *Curi Bio, Seattle, WA, USA*
Berry, Bonnie - *Curi Bio, Curi Bio, Seattle, WA, USA*
Gray, Kevin - *Curi Bio, Curi Bio, Seattle, WA, USA*
Kharoufeh, Samir - *Curi Bio, Curi Bio, Seattle, WA, USA*
Macadangdang, Jesse - *Curi Bio, Curi Bio, Seattle, WA, USA*
Worthen, Christal - *Curi Bio, Curi Bio, Seattle, WA, USA*

Abstract: Model systems that accurately recapitulate healthy and diseased function in a dish are critical for the development of novel therapeutics. For cardiac and skeletal muscle diseases, direct assessment of contractile output constitutes the most reliable metric with which to assess overall tissue function, as other 'proxy' measurements are poor predictors of muscle strength. 3D engineered muscle tissues (EMTs) derived from iPSCs hold great potential for modeling contractile function. However, the bioengineering strategies required to generate these predictive models are oftentimes out of reach for most investigators. Here, we have developed a platform and device that utilizes 3D EMTs in conjunction with a label-free magnetic sensing array. The platform enables facile and reproducible fabrication of 3D EMTs using virtually any cell source, and is coupled with a highly parallel direct measurement of contractile strength. This approach enables the stratification of healthy and diseased muscle phenotypes and facilitates dose-dependent compound safety and efficacy screening for evaluation of a drug's effect on contractile output. We will present a 3D model of Duchenne muscular dystrophy (DMD) that utilizes skeletal muscle EMTs formed from an isogenic pair of healthy and diseased cells. These constructs achieve robust twitch and tetanic responses upon stimulation. We then use these models to compare functional metrics of contractility such as force and fatigability over weeks or months. Our platform also

lends itself to the utilization of other optically-based assays such as calcium detection, which is a critical phenotype in diseases such as DMD where calcium handling is misregulated. We will also present data showing both acute and chronic drug toxicity in both cardiac and skeletal muscle EMTs including a drug (BMS-986094) that failed clinical trials due to unanticipated cardiotoxicity. These data demonstrate a first-and-only commercial platform for high-throughput assessment of 3D skeletal and cardiac muscle contraction with potential for widespread adoption within the drug development field.

Keywords: Cardiac Disease Models, 3D Muscle Organoids, Drug Discovery

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HUMAN XIST RNA ACTS EARLY TO CONDENSE ARCHITECTURE WHICH FACILITATES A-REPEAT DENSITY-DEPENDENT INITIATION OF GENE SILENCING

Valledor, Melvys - *Neurology, University of Massachusetts Medical School, Worcester, MA, USA*
Byron, Meg - *Neurology, University of Massachusetts Medical School, Worcester, MA, USA*
Carone, Dawn - *Department of Biology, Swarthmore College, Swarthmore, PA, USA*
Dumas, Breet - *Department of Medicine, Boston University Medical Center, Boston, MA, USA*
Hall, Lisa - *Neurology, University of Massachusetts Medical School, Worcester, MA, USA*
Lawrence, Jeanne - *Neurology, Pediatrics, University of Massachusetts Medical School, Worcester, MA, USA*

Abstract: XIST RNA triggers gene silencing chromosome-wide and transforms a euchromatic chromosome into a condensed Barr body. XIST is heavily studied in mouse ES cells, but here an inducible iPSC system allows analysis of initial steps in human chromosome silencing, revealing key points not known in either system. XIST RNA distribution was examined relative to biochemical and transcriptional changes directly within architecture of individual chromosome territories. Within a few hours of induction, XIST transcripts distribute as a large “sparse zone” and a smaller “dense zone”, which, importantly, exhibit different effects on chromatin. Very sparse transcripts immediately trigger bright staining for H2AK119ub and CIZ1, a structural matrix protein. In contrast, H3K27me3 enrichment comes hours later and is much more restricted to the smaller dense RNA zone, which enlarges as the chromosome condenses. Importantly, silencing of several genes examined occurred well after architectural condensation, suggesting a possibly separable step. Surprisingly, we show the small A-repeat fragment of XIST can alone silence endogenous genes; however, results indicate this requires high local RNA density for effective histone deacetylation. Results support a concept whereby XIST RNA acts directly to condense the chromosome territory, comprised largely of non-coding DNA, which facilitates a required step to initiate gene silencing by the A-repeat. Hence, compacted architecture is not a consequence of collective gene silencing, but an early step required for chromosome-wide gene silencing.

Funding Source: NIH—grants R01R35GM122597, R01HD091357, and R01HD094788 to J.B.L. and F32 AG056131-01 to MV
Keywords: XIST A-Repeat, CIZ1, H3K27, UbH2A, Transcription, Heterochromatin, Barr body

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HEPARAN SULFATE REGULATES CELL FATE DECISIONS OF HUMAN EMBRYONIC STEM CELLS

Syangtan, Deepsing - *Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA*
Al-Mahbuba, Deena - *Chemistry, MIT, Cambridge, MA, USA*
Masuko, Sayaka - *Chemistry, MIT, Cambridge, MA, USA*
Kiessling, Laura - *Chemistry, MIT, Cambridge, MA, USA*

Abstract: 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 embryoid body formation assays and directed differentiation assays, we found that the differentiation potential of EXT1-/- cells to mesoderm and endoderm lineages are severely compromised. Moreover, EXT1-/- cells can differentiate to early 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, Mesendoderm, Ectoderm

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DEFINING THE ROLE OF KDM6B IN AUTISM SPECTRUM DISORDERS USING HUMAN PLURIPOTENT STEM CELL-DERIVED MICROGLIA

Gulesserian, Harout - *Neurobiology, University of California, Los Angeles, CA, USA*
Novitch, Bennett - *Neurobiology, University of California, Los Angeles, CA, USA*
Buth, Jessie - *Neurobiology, University of California, Los Angeles, CA, USA*
Malone, Cindy - *Biology, California State University Northridge, CA, USA*

Abstract: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder that currently has no cure. ASD patients present with abnormalities in social interaction and behavior. Studies indicate ASD patient brains display increased activation of microglia, deficits in synaptic connections, and a reduction in functional connectivity. However, the specific mechanisms of how this may occur remain unclear. One promising theory (due to its targetability) is that dysfunctional microglia may drive or perpetuate ASD pathology. This could occur through many aspects of microglial function. At rest, microglia are involved in immune surveillance and homeostatic regulation of synaptic connectivity. In response to damage in the brain, microglia can become activated, proliferate, migrate to the site of injury, secrete pro- or anti-inflammatory cytokines, and phagocytose pathogens/debris. Many genes have been associated with ASD; however, none are specifically expressed in microglia alone. Lysine demethylase 6 beta (KDM6B) is a high-confidence ASD risk gene that modifies chromatin by removing repressive methyl groups from tri- or di- methylated lysine 27 on histone 3, which results in increased

gene expression. Prior findings indicate that KDM6B is a modulator of neuroinflammation and is particularly enriched in microglia. Notably, we hypothesized a loss of KDM6B in microglia would result in an increased inflammatory response, altered cytokine secretion, and changes in phagocytosis which may lead to ASD pathology. To date, many studies on human neurodevelopment and neuroinflammation have relied on mouse models but failed to recapitulate the specific complexity of the human brain. For this reason, we used human pluripotent stem cell (hPSC)-derived microglia to identify whether chemical inhibition or CRISPR mutations in KDM6B would alter microglia functions. We measured changes in microglial: (1) proliferation, (2) motility/morphology, (3) migration, and (4) phagocytosis. Together, the data suggests loss of KDM6B function seemingly leads to aberrant microglial activation with consequences on neural network connectivity and function.

Keywords: Autism Spectrum Disorder, KDM6B, Microglia

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CELLULAR SYSTEMS FOR THE IDENTIFICATION OF PATHOLOGICAL PATHWAYS IN HUNTINGTON'S DISEASE

Oweis, Walaa Z. - *Genetics, The Hebrew University, Jerusalem, Israel*
Sorek, Matan - *Genetics, The Hebrew University of Jerusalem, Israel*
Nissim-Rafinia, Malka - *Genetics, The Hebrew University of Jerusalem, Israel*
Meshorer, Eran - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Huntington's disease (HD) is an incurable, neurodegenerative disorder. While the genetic mutation causing HD has been identified over 25 years ago, the pathological mechanisms underlying HD are still not clear. The abnormal number of tri-nucleotide CAG repeats in the Huntingtin (HTT) gene, which encodes a polyglutamine (polyQ) expansion, leads to HTT protein misfolding and aggregate formation. To elucidate the pathological pathways involved in aggregates formation and clearance in HD, we developed a human cellular model for polyQ-related disorders, including Huntington's Disease (HD). We ectopically expressed the first 21 exons of the Huntingtin (HTT) gene, containing the pathogenic variant of 105Qs (GFP-105Q) in corrected iPSC-derived HD patient cells. In addition, we expressed 18Q-GFP in iPSC-derived HD patient cells containing 180Q and enriched for an 180Q-iPSC population with polyQ aggregates (GFP-180Q). We then differentiated both 180Q-GFP and 105Q-GFP iPSCs into neuronal progenitor cells (NPCs). Using several consecutive rounds of sorting (FACS), we were able to isolate pure populations of polyQ-aggregates-containing human NPCs. By comparing gene expression profiles using RNA sequencing between GFP-105Q and GFP-180Q with and without aggregates, we identified several genes and pathways which are uniquely activated or deactivated in the polyQ-aggregates-containing cells. Among the several factors was ATF3 which was highly induced in NPCs containing aggregates. knocking out ATF3 in our neuronal progenitors delayed the formation of polyQ-aggregates, suggesting that ATF3 is directly involved in their formation. ChIP-seq experiments of ATF3 revealed exclusive binding to several promoters of inflammatory genes which were activated in aggregates-containing cells. Taken together, our results highlight ATF3 as an activator of inflammatory genes in polyQ-aggregates-containing cells.

Keywords: Huntington's disease, Aggregates, Induced pluripotent stem cells (iPSCs)

TOPIC: CARDIAC

825

GENERATION OF MATURE COMPACT VENTRICULAR CARDIOMYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

Funakoshi, Shunsuke - *Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto, Japan*
Keller, Gordon - *McEwen Stem Cell Institute, McEwen Stem Cell Institute, Toronto, ON, Canada*

Abstract: Compact cardiomyocytes that make up the ventricular wall of the adult heart represent an important therapeutic target population for modeling and treating cardiovascular diseases. Here, we established a differentiation strategy that promotes the specification, proliferation and maturation of compact ventricular cardiomyocytes from human pluripotent stem cells (hPSCs). The cardiomyocytes generated under these conditions display the ability to use fatty acids as an energy source, a high mitochondrial mass, well-defined sarcomere structures and enhanced contraction force. These ventricular cells undergo metabolic changes indicative of those associated with heart failure when challenged in vitro with pathological stimuli and were found to generate grafts consisting of more mature cells than those derived from immature cardiomyocytes following transplantation into infarcted rat hearts. hPSC-derived atrial cardiomyocytes also responded to the maturation cues identified in this study, indicating that the approach is broadly applicable to different subtypes of the heart. Collectively, these findings highlight the power of recapitulating key aspects of embryonic and postnatal development for generating therapeutically relevant cell types from hPSCs.

Keywords: cardiac maturation, cell transplantation, disease model

TOPIC: EARLY EMBRYO

827

TWO WAVES OF H3K9ME3 ALLOCATIONS ENSURE RETROTRANSPOSON SILENCING IN HUMAN PREIMPLANTATION EMBRYOS

Xu, Ruimin - *School of Life Sciences and Technology, Tongji University, Shanghai, China*
Li, Sen - *Reproductive Medicine Center, Guangdong Second Provincial General Hospital, Guangzhou, China*
Wu, Qiu - *School of Life Sciences and Technology, Tongji University, Shanghai, China*
Li, Chong - *School of Life Sciences and Technology, Tongji University, Shanghai, China*
Jiang, Manxi - *Reproductive Medicine Center, Guangdong Second Provincial General Hospital, Guangzhou, China*
Guo, Lei - *Reproductive Medicine Center, Guangdong Second Provincial General Hospital, Guangzhou, China*

Abstract: H3K9me3 is a hallmark of heterochromatin and is important for cell fate specification. However, it remains unknown how H3K9me3 is reprogrammed during human embryo development. Here, we profiled genome-wide H3K9me3 in human oocytes and early embryos and discovered two waves of H3K9me3 deposition in long terminal repeats (LTRs) during peri- and post-zygotic genome activation (ZGA). We found that peri-ZGA-specific H3K9me3 was temporarily established on enhancer regions and may be regulated by several transcription activators, including DUXA. Moreover, TRIM28 and KRAB-ZNF transcription factors might be responsible for the establishment of post-ZGA-specific H3K9me3, and LTRs silenced at the post-ZGA stage by H3K9me3 are more likely to function as alternative promoters during ZGA. Finally, we observed the existence of H3K4me3/H3K9me3 and H3K4me4/H3K27me3 bivalent chromatin domains in blastocysts, priming for lineage differentiation. Therefore, our data unveiled that the epigenetic switch from DNA methylation to H3K9me3 ensures the silencing of activated retrotransposons in human embryos.

Keywords: human preimplantation embryo, H3K9me3, LTR

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

829

IN VITRO MODELING OF HUMAN VASCULATURE USING BLOOD VESSEL ORGANOIDS PROVIDES A ROBUST PLATFORM FOR STUDYING BLOOD VESSELS IN NORMAL AND PATHOLOGICAL CONDITIONS

Marchetti, Valentina - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Dhillon, Ishpreet - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Pippard, Sara - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Wagey, Ravenska - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Wimmer, Reiner - *Research and Development, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Penninger, Josef - *Medical Science, University of British Columbia, Vancouver, BC, Canada*
Conder, Ryan - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Eaves, Allen - *Corporate, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*

Abstract: Blood vessels are a fundamental part of all organ systems and have critical roles in multiple diseases, including diabetes, atherosclerosis and cancer. The blood vasculature is composed of endothelial cells that form luminal tubes, perivascular cells and a basement membrane that provides vessel support. In vitro models of vasculature involve co-culturing endothelial cells with pericytes but do not fully recapitulate their three dimensional organization, and the complex cell signaling observed in vivo. A novel culture system where human pluripotent stem cells (hPSC) derived blood vessel organoids are used to model the structural and functional features of blood vasculature was reported. Based on these publications, we have developed the STEMdiff™ Human Blood Vessel Organoid (BVO) Kit to enable robust and reproducible generation of blood vessel organoids in culture. Aggregates derived from hPSC lines are first generated in STEMdiff™ BVO Aggregation Medium followed by generation of mesoderm and vascular progenitor cells using STEMdiff™ BVO Mesoderm and Vascular Induction Media, respectively. Vascular aggregates sprout into vascular networks and mature into stable blood vessels when grown within the extracellular matrix in STEMdiff™ BVO Maturation Medium. PSC-derived blood vessel organoids contain endothelial cells (60% CD31+CD144+ n=4-6) and pericytes (30% CD140b+, n=4-6), and exhibit collagen IV deposition after 10 days in culture. Confocal microscopy revealed the formation of a complex network of tubes composed of CD31+ cells covered by pericytes and smooth muscle cells. Vascular networks were exposed to high glucose diabetic-mimicking conditions in vitro and a series of compounds known to control the diabetic microangiopathy was applied. The deposition of the extracellular matrix was evaluated. Increased expression of collagen IV was detected in “diabetic” vascular networks compared to controls. Treatment with ɣ-secretase or forskolin decreased deposition of collagen IV to level similar to control organoids. These data demonstrate that the STEMdiffTM Blood Vessel Organoid Kit supports reproducible generation of blood vessel organoids from hPSCs and may be used to model both normal and diseased blood vessels in vitro

Keywords: Blood Vessels, Organoids, Vasculopathy

TOPIC: EYE AND RETINA

831

DECODING TRANSCRIPTIONAL RESPONSE OF STEM CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS AFTER SUBRETINAL TRANSPLANTATION AT SINGLE CELL RESOLUTION

Parikh, Bhav H. - *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore*
Blakeley, Paul - *Ophthalmology, National University of Singapore, Singapore*
Kakkad, Regha - *Ophthalmology, National University of Singapore, , Singapore*
Liu, Zengping - *Ophthalmology, National University of Singapore, Singapore*
Lingam, Gopal - *Ophthalmology, National University of Singapore, Singapore*
Hu, Tim - *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore*
Su, Xinyi - *Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore*

Abstract: Visual dysfunction in age-related macular degeneration (AMD) is associated with the degeneration of retinal pigmental epithelium (RPE). Several ongoing clinical trials are testing cell replacement therapy as means to halt AMD progression, using human pluripotent stem cell (hPSC)-derived RPEs. Although grafted cells pre-transplantation are routinely characterized, it is unknown how these cells respond transcriptionally after transplantation. Therefore, we aim to dissect the single cell transcriptomic changes in hPSC-RPEs after transplantation. For this purpose, two sources of hPSCs, embryonic (ESCs) and induced (iPSCs), were differentiated to generate RPEs. These cells, at 1-month-old, resembled its native counterparts by expressing RPE signature markers, secreting cytokines in a polarized fashion, and demon-

strating barrier function and active phagocytosis. The hPSC-RPEs were then either grown further for a month in-vitro or subretinally transplanted into immunocompetent Dutch Belted rabbits and followed-up for an additional month. The transplanted hPSC-RPEs successfully xenografted subretinally into immunocompetent hosts and displayed maintenance of overlying retinal structure, and retinal function. There was absence of adverse reactions against both sources of RPE. At 2 months, both in-vitro and in-vivo transplanted cells were harvested for single cell RNA sequencing (scRNA-seq). ScRNA-seq of in-vitro RPE cultures from both sources revealed a similar heterogenous population consisting of a spectrum of varying maturity states, from progenitor to late RPE. Profiling of RPEs after subretinal transplantation unravelled robust maturation in vivo towards late RPE state. In conclusion, we generated high quality, functional RPEs from two different hPSC sources and demonstrated subretinal transplantation of these cells into an immunocompetent host without an adverse reaction, highlighting hPSCs as a promising cell source to treat AMD. Furthermore, scRNA-seq performed on subretinally transplanted hPSC-RPEs uncovered the favorable maturation of a heterogenous in-vitro RPE population towards a homogenous adult RPE state. Overall, our findings provide a high-resolution perspective on a stem cell-based product intended for future clinical use.

Keywords: Age related macular degeneration, Single-cell RNA-sequencing, Induced pluripotent stem cells

TOPIC: GERMLINE

833

OPTIMISING A NOVEL DIFFERENTIATION PROTOCOL FOR GONADAL AND EARLY TESTICULAR ORGANOIDS

Pachernegg, Svenja - *Reproductive Development Group, Murdoch Children's Research Institute, Parkville, Australia*
Robevska, Gorjana - *Reproductive Development Group, Murdoch Children's Research Institute, Parkville, Australia*
Charitakis, Natalie - *Transcriptomics and Bioinformatics Group, Murdoch Children's Research Institute, Parkville, Australia*
Sinclair, Andrew - *Reproductive Development Group, Murdoch Children's Research Institute, Parkville, Australia*
Ayers, Katie - *Reproductive Development Group, Murdoch Children's Research Institute, Parkville, Australia*

Abstract: Reproductive development can be disrupted by both environmental and genetic factors, with Differences of Sex Development affecting an about 1.7% of babies. There is an urgent need for an in vitro model of human embryonic gonadal development to study these disorders. We have addressed this by establishing a world-first protocol to differentiate human induced pluripotent stem cells (iPSCs) into early gonadal cells cultured as testis-like organoids. Our stepwise differentiation protocol uses small molecules to mimic developmental signalling, inducing iPSCs to develop into the bipotential gonad by day 7. Aggregating and culturing these cells as 3D organoids results in significant testis gene expression by day 21. In these organoids, cells expressing testis markers reside within tissue structures delineated by basement membrane. These "tube-like structures" are reminiscent of cord-like assemblies in re-aggregated mouse testes. Transcriptomic profiling of our organoids using single cell RNA sequencing (scRNA-seq) shows clusters with gonadal and reproductive tissue identities, and distinct testicular cell lineages, which overlap with those found in human fetal gonads. We are now optimising the existing protocol to increase the generation of these tube-like structures within the organoids, and to enhance the proliferation and differentiation of more mature testicular cell types.

Funding Source: This project was funded by two NHMRC Ideas grants (K.L.A., APP1156942 and APP2012250) and Program grant (A.H.S., APP1074258) from the National Health and Medical Research Council (NHMRC), Australia.
Keywords: Disorders of Sex Develoment, gonads, testis

TOPIC: LIVER

835

SPHEROIDS PROMOTE MORE EFFECTIVE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO HEPATOCYTES

Tomizawa, Minoru - *Department of Gastroenterology, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan*
Shigeta, Midori - *Department of Pediatrics, National Hospital Organization, Shimoshizu Hospital, Yotsukaido City, Japan*
Shinozaki, Fuminobu - *Department of Radiology, National Hospital Organization, Shimoshizu Hospital, Yotsukaido City, Japan*
Tanno, Hirokazu - *Department of Neurosurgery, National Hospital Organization, Shimoshizu Hospital, Yotsukaito City, Japan*

Abstract: Hepatocytes can be used in liver transplantation; however, their isolation from the human liver is technically difficult. Human induced pluripotent stem (iPS) cells can differentiate into hepatocytes in a medium lacking glucose (hepatocyte selection medium, HSM) supplemented with 1mM lactate calcium in conventional plates or dishes as flat cultures, but the differentiation might remain incomplete, forming immature cells. We expected spheroid cultures to promote iPS cells differentiation into hepatocytes more effectively than flat culture. iPS cells were cultured in NutriStem hPS XF (Biological Industries, Cromwell, CT), a medium to maintain an undifferentiated state, on the bottom of 15 ml conical tubes coated with poly(2-hydroxyethyl methacrylate). The cultured cells formed spheroids. Media were changed to HSM supplemented with 1mM calcium lactate. After four days, the media was changed to NutriStem hPS XF, and cells were cultured for seven days. Meanwhile, iPS cells were cultured on a 6-well plate coated with Matrigel (Flat) with 5 × 105 cells in each tube or well. After isolating total RNA, cDNA was synthesized and subjected to real-time quantitative PCR. Alpha-fetoprotein and albumin were used as markers of hepatocytes. Expression levels of AFP in flat and spheroid cultures were 2.9 ± 0.3 (mean ± standard deviation) and 20.8 ± 5.6, respectively. Expression levels of albumin in flat and spheroid cultures were 5.7 ± 1.0 and 14.8 ± 2.9, respectively. As expected, iPS cells differentiated into hepatocytes in spheroids more effectively than conventional flat culture. Our next step would be to confirm the hepatocyte differentiation of the obtained cells in spheroids with immunostaining and analysis of hepatocyte-specific function, such as gluconeogenesis and urea cycle.

Keywords: induced pluripotent stem cells, hepatocyte, spheroids

TOPIC: MUSCULOSKELETAL

837

GNL3/NUCLEOSTEMIN AND THE PATHOGENESIS OF KNEE OSTEOARTHRITIS

Seah, Matthew - *Department of Surgery, University of Cambridge, UK*
Birch, Mark - *Department of Surgery, University of Cambridge, UK*

Abstract: Genome-wide association studies have implicated several genes in the development of knee osteoarthritis (OA). Among these candidate genes is GNL3, which encodes the protein nucleostemin which is found in stem cells. It functions to regulate cell cycle progression and tissue regeneration but its role in the joint is unknown. We aim to characterise the effects of GNL3 deletion in a mouse model, and corroborate the findings with knockdown experiments in human mesenchymal stromal cells (MSCs) and human articular chondrocytes to study the role GNL3 has in the pathogenesis of OA. The effect of GNL3 deletion in a mouse model was studied using histology (to assess cartilage thickness), microCT for bone morphometry, and scanning electron microscopy to further characterise the subchondral structure and perform elemental analysis. These were compared to age-matched controls. siRNA-mediated knockdown of GNL3 in human MSCs and chondrocytes was performed to examine the effects on chondrogenesis-related genes using RT-PCR. GNL3 heterozygote mouse knees had thinner layers of articular cartilage compared to aged-matched controls. They also had thicker trabeculae, and greater spacing between the trabeculae. They showed significantly reduced alkaline phosphatase staining, suggesting reduced osteogenic activity from osteoblasts. Knockdown of GNL3 using siRNA in MSCs and articular chondrocytes did not significantly alter the mRNA expression of chondrogenic genes such as COL2A1, but there was significant upregulation of the metalloproteinase ADAMTS4. The increase in ADAMTS4 expression might be implicated in the pathogenesis of OA, and GNL3 may represent a local target in disease-modifying therapies. However, as GNL3 is expressed widely by other cell types, further work should be done to investigate the role of GNL3 deletion in other cell types which may have a role in the endogenous repair of damaged cartilage.

Funding Source: MS is funded by a grant from the Wellcome Trust (PhD Programme for Clinicians)
Keywords: nucleostemin, osteoarthritis, GNL3

TOPIC: NEURAL

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INHIBITION OF REACTIVE ASTROCYTES BY MESENCHYMAL STEM CELL-DERIVED NEURAL PROGENITORS SUGGEST POTENTIAL THERAPEUTIC MECHANISM OF ACTION IN MULTIPLE SCLEROSIS

Carlson, Alyssa - *Regenerative Neurology, Tisch MS Research Center of New York, NY, USA*
Wollowitz, Jaina - *Regenerative Neurology, Tisch MS Research Center of New York, NY, USA*
Sadiq, Saud - *Regenerative Neurology, Tisch MS Research Center of New York, NY, USA*
Harris, Violaine - *Regenerative Neurology, Tisch MS Research Center of New York, NY, USA*

Abstract: Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the CNS. The progressive phase of MS is characterized by chronic demyelination, microglial activation, reactive astrogliosis, and axonal loss leading to a steady worsening of neurologic function with limited therapeutic options. To address this unmet medical need, we developed a novel cellular therapy utilizing autologous bone marrow mesenchymal stem cell-derived neural progenitor (MSC-NP) cells administered in multiple intrathecal injections in patients with progressive MS. A recent Phase I trial demonstrated safety, improved neurologic function in some patients, and reduced levels of the biomarker CCL2 in cerebrospinal fluid. The therapeutic action of MSC-NPs is associated with secreted immunomodulatory and trophic factors that act in a paracrine manner to influence tissue repair. Because pro-inflammatory neurotoxic reactive astrocytes secrete CCL2 and play a role in promoting MS disease pathology and progression, we hypothesized that factors released by MSC-NPs may influence astrocyte reactivity. To model reactive astrogliosis, we differentiated human iPS cells into astrocytes as defined by characteristic morphology and marker expression including GFAP, SLC1A3, and Vimentin. Resting astrocytes were stimulated with TNFa, IL-1a, and C1q to mimic A1-like reactive astrocytes and expression of A1 markers were determined by PCR and ELISA. MSC-NPs were derived according to standardized manufacturing protocols from bone marrow of 10 individual donors with MS taking part in a phase II clinical trial. Conditioned media from MSC-NPs (NPCM) was collected after 2 to 3 days in culture, and unconditioned media (NPMM) was used as a control. Astrocytes stimulated in the presence of NPCM resulted in a significantly decreased expression of activated astrocyte markers, including VCAM1, CD274, C3, CCL5 and IL-6. The NPCM-mediated decrease of A1 marker expression was associated with inhibition of a neurotoxic phenotype in astrocytes in vitro. These results suggest that MSC-NP-based cell therapy may impact clinical disease progression through an astrocyte-mediated mechanism, and reveal candidate biomarkers of therapeutic potency in MS.

Keywords: multiple sclerosis, mesenchymal stem cells, astrocytes

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EPITHELIAL CELL DIVERSIFICATION IN THE PRENATAL HUMAN CHOROID PLEXUS REVEALED BY A STEM CELL MODEL AND SINGLE CELL RNA SEQUENCING

Masters, Haley - *Developmental and Cell Biology, University of California, Irvine, Mission Viejo, CA, USA*
Jaime, Olga - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Johnson, Brett - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Kessenbrock, Kai - *Biological Chemistry, University of California, Irvine, CA, USA*
Martel, Cristina - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Monuki, Edwin - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Neel, Michael - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Nguyen, Quy - *Biological Chemistry, University of California, Irvine, CA, USA*
Nie, Qing - *Developmental and Cell Biology, University of California, Irvine, , CA, USA*

Shi ru fung, Pamela - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Tran, Benjamin - *Developmental and Cell Biology, University of California, Irvine, CA, USA*
Tu, Christina - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Wang, Shuxiong - *Developmental and Cell Biology, University of California, Irvine, CA, USA*

Abstract: As the interface between the blood and cerebrospinal fluid, the choroid plexus (ChP) mediates body-brain homeostasis and has strategic potential for regenerative medicine. Despite this, little is known about the human ChP, ranging from functions, mechanism, and developmental pathways, or its functional unit, the epithelial cells (CPECs), which have been considered a uniform cell type. Based on an earlier proof-of-concept method, we devised a simple, efficient, and scalable protocol for CPEC differentiation from human pluripotent stem cells. Derived CPECs (dCPECs) developed canonical CPEC properties and functions in the absence of mesenchymal elements. In addition, dCPECs displayed diverse multiciliated phenotypes, that impacted Aβ uptake in a reciprocal manner, and paralleled those in perinatal human tissues. Single cell dCPEC transcriptomes correlated well with a human organoid and a fetal CPEC transcriptome, and pseudotemporal analyses revealed the direct dCPEC origin from neuroepithelial cells that also produced neurons and neural progenitors. Time course bioinformatic tools, in addition to lineage and cell cycle analyses, identified dCPEC diversifications at early and later postmitotic stages with subtypes enriched for anabolic-secretory (type 1a), catabolic-absorptive (type 1b), and ciliogenesis pathways (type 2). Ontogenesis of these subtypes was then validated in independent derivations and perinatal tissues. These findings establish a robust human CPEC model system for basic studies, disease modeling, and regenerative medicine applications while revealing cilia and subtype diversifications in postmitotic CPECs during prenatal human development.

Keywords: Choroid Plexus, human stem cell model, single cell RNA sequencing

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ENGINEERING HUMAN NEURAL ORGANOIDS TO EXPLORE DEVELOPMENTAL NEUROTOXICITY

Wu, Xian - *DNTP, NIEHS, Durham, NC, USA*
Dixon, Darlene - *DNTP, NIEHS, Durham, NC, USA*
Tokar, Erik - *DNTP, NIEHS, Durham, NC, USA*

Abstract: Modeling brain development and function is challenging due to the complexity of the organ. Recent advances in human pluripotent stem cell (PSC)-derived brain-like organoids provide new tools to study the human brain. These 3D models together with 2D cellular assays have the potential to enhance our understanding of the mechanisms of developmental neurotoxicity (DNT) during the early stages of neurogenesis and offer a rapid, cost-effective approach for assessing chemical safety. Arsenic (As) is a widespread environmental contaminant. Exposure to As is associated with DNT-related diseases. However, mechanisms of As on DNT are not well-defined. Here, we used 3D PSC-derived embryoid bodies (EBs) to recapitulate events involved in early embryogenesis, and EB-derived neural organoids to mimic neural development in vivo. 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 early embryonic development genes PAX6, SOX1, COL2A1. Histological staining of As-treated EBs showed neural rosette structure disruption. Organoids showed VIMENTIN+ astrocytes and NESTIN+ neural SCs, confirming cerebral phenotype. Ingenuity pathway analysis (IPA) of RNAseq data from organoids indicated CREB signaling was activated during neural maturation (day 7 to day 40) while pluripotency signaling was suppressed. The IPA-identified top 5 pathways affected by As treatment were CREB signaling in neurons, neuronal synapse pathway, GABA receptor pathway, synaptogenesis signaling and axonal guidance signaling. GO enrichment analysis found G-protein signaling on the cell membrane was suppressed and WNT signaling pathway was inhibited by As treatment. RNAseq analyses were confirmed by real-time qPCR, which also found As-inhibited expression of markers for mature neural cells (MAP2, vGLUT2) and astrocytes (GFAP). Inhibition of neuron maturation was also confirmed in a neurite outgrowth 2D assay that mimics nerve growth and axon pathfinding in vivo and in the disrupted neural rosette and neuropil structures in day 40 organoids. In conclusion, the results described herein show that this EB and neural organoid 3D model can provide valuable insights into the cellular events and molecular mechanisms of As-induced DNT.

Keywords: Neural organoid, Developmental neurotoxicity, Embryoid bodies

845

CROSSING THE HUMAN BLOOD-BRAIN BARRIER WITH ADENO-ASSOCIATED VIRUSES: A STEM CELL-DERIVED IN VITRO MODEL AS SCREENING PLATFORM FOR AAV VARIANTS.

Nikolakopoulou, Polyxeni - *Neuroscience, Karolinska Institutet, Solna, Sweden*
Voulgaris, Dimitrios - *Department of Protein Science, KTH Royal Institute of Technology, Solna, Sweden*
Stergiou, Eleni - *Department of Neuroscience, Karolinska Institutet, Solna, Sweden*
Herland, Anna - *Department of Neuroscience, Karolinska Institutet, Solna, Sweden*

Abstract: Treating human central nervous system (CNS) disorders remains an unmet need. The blood-brain barrier (BBB) protects the human brain from invaders via the circulation; however, it also hinders drug delivery and disease treatment. Therefore, it is crucial to identify novel agents that can cross the BBB without affecting its integrity. To this end, adeno-associated viruses (AAVs) have emerged as a putative option for CNS therapy development. Animal studies show that AVVs can act as CNS transducers after efficient barrier crossover; nevertheless, virus crossover and transduction efficiency are variant- and species-dependent. Trafficking of AAV9 is more efficient than AAV2 when directly compared using primary human endothelial cells. As also shown in animal studies, AAV9 successfully penetrates the barrier and transduces human astrocytes. Interestingly, AAV-PHP.eB -a recently identified variant of AAV9- shows higher efficiency than all studied variants to penetrate the BBB. Up to date, these studies are performed in mice, thereby posing translatability issues. Interestingly, evidence shows that AAV-PHP.eB transport is mediated via the Ly6a receptor in mice, a gene that is not expressed in humans. Here, we used human induced pluripotent stem cell (hiPSC)-derived BBB-like cells and hiPSC-derived astrocytes (hiAstros) to mimic the microenvironment of the human neurovascular unit (NVU) and assess the potential of three different AAV variants (AAV2, AAV9 and AAV-PHP.eB) to cross the human BBB. The high transendothelial resistance (TEER values ≈4000 Ω*cm2) of our barrier highlights the system's in vivo-like character, thus

enhancing the predictivity of the observed transport events. Our data show that the studied AAVs show differential BBB transport and astrocyte transduction patterns. Moreover, we provide evidence that AAV-PHP.eB penetrates the barrier via receptor-mediated transcytosis and not barrier breakdown using a tight BBB model. Lastly, our NVU-on-a-chip culture system utilizes microfluidics to recapitulate the microphysiological properties of the brain tissue and increase the biomimicry of our model. In a nutshell, the hiPSC-based in vitro model may serve as a patient-oriented screening platform for AAV variants and evaluate their potential as gene therapy vectors for human CNS disease.

Keywords: Blood-Brain Barrier, Adeno-associated viruses, Human induced pluripotent stem cells

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17β-ESTRADIOL TREATMENT ENHANCED THE NEURONAL EXCITABILITY AND NEURITE BRANCHING OF HUMAN IPS-BASED ALZHEIMER'S DISEASE MODEL

Supakul, Sopak - *Physiology, Keio University School of Medicine, Tokyo, Japan*
Maeda, Sumihiro - *Physiology, Keio University School of Medicine, Tokyo, Japan*
Okano, Hideyuki - *Physiology, Keio University School of Medicine, Tokyo, Japan*

Abstract: Alzheimer disease (AD) is a neurodegenerative disease associated with cognitive decline. While several clinical trials developed based on familial AD models have failed to achieve the pleasant outcomes, it is believed that the heterogenous pathogenesis of sporadic AD (sAD) cases led to the variable drug responses. Therefore, the generation of the robust disease model of sAD as a platform for understanding AD heterogeneity has been strongly suggested. Here, we generated the iPSCs-derived cortical neurons including the excitatory and inhibitory neurons using the newly developed induction method for feeder-free iPS. The donors are comprised of healthy control, familial AD, and sAD cases. These iPSC-derived neurons demonstrated the disease phenotypes of AD such as increased amyloid-beta (Aβ) secretion evaluated by ELISA and neuronal hyperactivation evaluated by calcium imaging using the Fluo-8 indicator. In addition, we evaluated the non-cell autonomous effect of sex dimorphism coming from sex hormone by treating the differentiated neurons with 17β-estradiol. As an acute effect, 17β-estradiol treatment for 15 minutes increased neuronal activity. While the treatment for 4 days promoted neurite branching as a chronic effect. These findings indicated that (i) the disease modeling of AD from hiPSC can be achieved by this newly established feeder-free induction method, (ii) hiPSC-derived AD neurons respond to sex steroid hormones, (iii) the current AD models enable the investigation of the variability of 17β-estradiol treatment responses among patients, and may contribute to the development of the future clinical trial on hormone replacement therapy among AD patients.

Keywords: Alzheimer's Disease, Induced Pluripotent Stem Cells (iPSCs), Hormonal Therapy

TOPIC: PANCREAS

849

NOTCH GOVERNS CELL FATE CHOICES VIA HES1 USING A DEEP GENE REGULATORY NETWORK ARCHITECTURE

Mjoseng, Heidi K - *Palle Serup Lab (reNEW), University of Copenhagen, Denmark*
Honnens de Lichtenberg, Kristian - *reNEW, University of Copenhagen, Denmark*
Raineri, Silvia - *reNEW, University of Copenhagen, Denmark*
Petersson, Eva - *reNEW, University of Copenhagen, , Denmark*
Funa, Nina - *reNEW, University of Copenhagen, Denmark*
Schreiber, Valérie - *Institut de Génétique et de Biologie Moléculaire et Cellulaire, University of Strasbourg, France*
Nissen, Silas - *Niels Bohr Institute, University of Copenhagen, Denmark*
Lee, Kihyun - *Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Pers, Tune - *Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark*
Trusina, Ala - *Niels Bohr Institute, University of Copenhagen, Denmark*
Ferrer, Jorge - *Section on Epigenetics and Disease, Imperial College London, UK*
Huangfu, Danwei - *Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Gradwohl, Gerard - *Institut de Génétique et de Biologie Moléculaire et Cellulaire, University of Strasbourg, Illkirch, France*
Serup, Palle - *reNEW, University of Copenhagen, Denmark*

Abstract: Notch signaling controls developmental cell fate choices in many organs including the pancreas. Using human embryonic stem cell (hESC)-derived pancreatic progenitor cells (hESC-PPs) as a model system, we have uncovered that Notch-induced HES1 represses differentiation-inducing gene regulatory networks (GRNs), through a "deep" GRN architecture. Transcriptomic profiles of wildtype and HES1-, NGN3-, and HES1/NGN3-deficient hESC-PPs together with determination of genomic HES1 binding locations showed that pancreatic endocrine and acinar fates are directly suppressed by HES1, not only through repression of the bHLH master regulator genes NGN3 and PTF1A, respectively, but also via direct repression of downstream NGN3 and PTF1A target genes. Moreover, the combined loss of HES1 and NEUROG3 led to marked upregulation of ATOH1 and ASCL1, two Hes1-bound, bHLH master regulator genes of non-pancreatic GRNs. Importantly, scRNA-seq revealed that ATOH1 and ASCL1 expression occurred in discrete cell populations not observed in wildtype cells. These populations had transcriptomic signatures indicating a mixed lineage with features from pancreatic and stomach/intestinal secretory lineages, suggesting that Hes1 and Neurog3 cooperatively suppress the emergence of inappropriate gene expression programs in pancreas progenitors. Notably, we found HES1 bound to many ATOH1 target genes in both hESC-PPs and in a cell line model of intestinal stem cells, suggesting that HES1 engage in a deep GRN architecture also in intestinal stem cells. Our results provide new insights into how Notch controls cell fate decisions that will aid directed differentiation of stem cells to clinically useful cell types.

Funding Source: The Novo Nordisk Foundation - reNEW grant number NNF21CC0073729.
Keywords: Pancreas, Notch, Gene regulation

TOPIC: PLURIPOTENT STEM CELLS

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DERIVATION OF HUMAN INDUCED PLURIPOTENT STEM CELL LINES FROM AFRICAN-RESIDENT POPULATION FOR DEMENTIA RESEARCH

Maina, Mahmoud - *Neuroscience, University of Sussex, UK, Damaturu, Nigeria*
Karch, Celeste - *Department of Psychiatry PhD, Washington University in St Louis, Washington, MO, USA*
Serpell, Louise - *Neuroscience, University of Sussex, Brighton, UK*
Wray, Selina - *Institute of Neurology, University College London, UK*

Abstract: Genetic studies have provided significant insight into the aetiology of dementia, especially Alzheimer's disease (AD). However, most of these studies have focussed on non-African residents, despite Africa's significant genetic diversity. Multiple studies have shown that ancestral background influences AD risk and pathways. For example, APOE ε4 is the strongest risk factor after ageing for late-onset AD in the European ancestry (EA) population, but not in the African ancestry population despite a high frequency of the APOE ε4 allele among Africans. Moreover, our current understanding of AD pathogenesis has come mainly from EA population using a number of models, including post-mortem tissue and human-induced pluripotent stem cell-derived neurons (hiPSC-neurons). Little is known on disease mechanisms from the African population partly due to the lack of access to these models from Africa. This raises the need for the inclusion of African models for AD research. hiPSC serve as an excellent model to understand AD and other neurological diseases in African residents due to its ability to capture the genetic diversity of the donor population. However, no hiPSC lines currently exist from the African-resident population. To address this major shortcoming, I have successfully grown fibroblasts from skin biopsies of Nigerian residents, which are currently being reprogrammed to hiPSC. Preliminary results show variation in the level of induction to hiPSC between fibroblasts from African and European volunteers. Ongoing work seeks to differentiate the hiPSC into neurons and eventually produce APOE ε4 isogenic lines that would collectively then be characterised using genome-wide association studies, RNA sequencing-based analysis, fluorescence imaging, and western blotting to fully understand the contribution of ancestry to hiPSC developmental and disease mechanism in relation to APOE ε4. Overall, for the first time, this work will result in the derivation of hiPSCs from the African-resident population, which can become a significant resource for the hiPSC field. In addition, it will reveal the impact of APOE ε4 allele in European hiPSC-neurons compared to the African hiPSC-neurons, helping to pinpoint the cellular and molecular features influencing AD development in these different ancestral lines.

Funding Source: University of Sussex Neuroscience Seed Funds and Alzheimer's Association
Keywords: Nigeria, Fibroblast, Genome-wide association studies, RNA sequencing

POSTER SESSION II: ODD

3:00 PM – 4:00 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: CARDIAC

503

BIOFABRICATION OF SMALL-DIAMETER BLOOD VESSELS

Cheng, Richard - *Surgery, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA, USA*
Miranda-Nieves, David - *Surgery, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA, USA*
Meissner, Torsten - *Surgery, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA, USA*
Haller, Carolyn - *Surgery, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA, USA*
Guenther, Axel - *Mechanical & Industrial Engineering, University of Toronto, Toronto, ON, Canada*
Chaikof, Elliot - *Surgery, Beth Israel Deaconess Medical Center / Harvard Medical School, Boston, MA, USA*

Abstract: Coronary artery bypass grafting is the most commonly performed cardiac surgery worldwide, but the standard of care remains the engraftment of patient-obtained veins that lead to stenosis or occlusion in nearly half of all cases after one year. This high failure rate has resulted in a demand for alternative strategies like tissue engineering which enable the formation of vessel-mimetic conduits with precise control over biomaterial composition and cell organization. Current approaches are limited by low-scale fabrication procedures and time-consuming methods that fail to generate sufficient quantities of patient-specific vascular cells. In response, we have developed a large-scale biofabrication process to quickly generate mechanically robust, free-standing, ultrathin collagen sheets as scaffold building blocks for vascular tissue engineering. Microfluidic extrusion, hydrodynamic focusing and strain-induced pulling is applied to a collagen solution, resulting in the formation of sheets with compatible physical dimensions (1.9 µm thick, 2.4 mm wide, and >230 mm long). Following the assembly of vascular smooth muscle cell-seeded sheets into tubular conduits via an automated rolling process and endothelialization, multiphoton z-stack imaging reveal an open-lumen vessel with uniform circumferential wall thickness conducive for unobstructed blood flow. Additionally, we have adopted a framework to differentiate human pluripotent stem cells (hPSCs) into vascular smooth muscle cells (vSMCs) and endothelial cells (ECs) under chemically defined conditions which serves to establish a rapid, efficient, and scalable cell source for tissue engineering. Plated hPSCs committed to vSMC fates with 95% efficiency in six days after modulating Wnt/β-catenin and TGF-β signaling pathways, whereas exposure to VEGF and forskolin followed by continued treatment with VEGF led to efficient induction of vascular ECs. Current efforts aim to establish these hPSC-derived vascular cells as suitable renewable cell sources for the engineered vascular conduit. Taken together, we report here a novel tissue engineering platform that enables the rapid and scalable production of "off-the-shelf" living engineered blood

vessels using vascular wall cells derived from hPSCs for prospective surgical engraftment.

Funding Source: American Heart Association (AHA) National Institutes of Health (NIH)
Keywords: Tissue Engineering, Microfluidics, Biofabrication

TOPIC: EPITHELIAL_GUT

505

FABRICATION OF AN ENVIRONMENTAL-CONTROLLED HUMAN-BASED COLON MICROPHYSIOLOGICAL SYSTEM

Hamel, Dimitri - *IRSD-INSERM U1220 & LAAS-CNRS, Toulouse, France*
Foncy, Julie - *Instrumentation Design and Characterization (I2C), LAAS-CNRS, Toulouse, France*
Ferrand, Audrey - *IRSD, Toulouse, France*
Malaquin, Laurent - *MNBT - ELIA Team, LAAS-CNRS, Toulouse, France*

Abstract: The development of in vitro artificial colonic microdevice, which reproduces more faithfully complex in vivo systems, are important tools to improve our understanding of the human gut physiology and pathologies. A main flaw of currently used flat culture systems in petri dishes is their lack of integration of the tissue topological aspects and their impacts on the tissue behavior and fate. In this context, synthetic in vitro models such as microphysiological systems (MPS) allow the control of specific parameters including tissue topology, rigidity and nutrients flow distribution. We developed a human-based colon microphysiological system, recapitulating more faithfully in vitro the 3D human colonic epithelium microenvironment including its topology and matrix support stiffness. This MPS integrates a microfluidic chamber that allows active control of both the apical/luminal and basal/stromal environment by accurate injection, but also sample recovery, as well as in situ imaging of tissue culture allowing live microscopy to evaluate cell migration, proliferation, apoptosis overtime. We established within the MPS the culture of colon epithelial cells derived from human colon organoids and could observed cell proliferation and the establishment of a polarized epithelium. This innovative device, combining 3D scaffold and microfluidic addressing, will allow a better understanding of the tissue (mechanobiology, architecture, functions...) and will be a useful tool to study microbiota, pathogens, and nutrients impacts on the colon epithelium as well as drug screening.

Funding Source: Plan Cancer “System biology” Université de Toulouse III French Région Occitanie HoliFAB - European Union’s Horizon 2020 MultiFAB - FEDER European Regional Funds and French Région Occitanie RENATECH
Keywords: Organ-on-chip, Biotechnology, Colonic epithelium & environment

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

507

VIRTUAL PROGRAMMING FOR DISSEMINATION OF STEM CELL AND TISSUE ENGINEERING RESEARCH

Tavakol, Daniel N - *Biomedical Engineering, Columbia University, New York, NY, USA*
Tavakol, Daniel - *Biomedical Engineering, Columbia University, New York, NY, USA*
Halligan, Susan - *Biomedical Engineering, Columbia University, New York, NY, USA*
Graney, Pamela - *Biomedical Engineering, Columbia University, New York, NY, USA*
Fleischer, Sharon - *Biomedical Engineering, Columbia University, New York, NY, USA*
Falcucci, Thomas - *Biomedical Engineering, Tufts University, Boston, MA, USA*
Zhuang, Richard - *Biomedical Engineering, Columbia University, New York, NY, USA*
Lock, Roberta - *Biomedical Engineering, Columbia University, New York, NY, USA*
Newman, Alexis - *Biomedical Engineering, Columbia University, New York, NY, USA*
Kaplan, David - *Biomedical Engineering, Tufts University, Boston, MA, USA*
Vunjak-Novakovic, Gordana - *Biomedical Engineering, Columbia University, New York, NY, USA*

Abstract: With the disruption of normal life due to the COVID-19 pandemic, virtual programming arose to provide much needed scientific dissemination and community building under rather challenging conditions. In person conferences, meetings, and faculty visits were cancelled. In the early weeks of the pandemic, we sought to bring together the tissue engineering community with a set of seminars, officially known as the “Tissue Talks” series, by providing an avenue where leaders in the field could present about their ongoing work and provide perspective on progress in our community. With what started out as a small initiative to help spread ongoing progress in the field, the series quickly spread all over the world, with up to 1000 individuals tuning in for the talks each week. Here, we describe the overall aims of using virtual programming to bring together the tissue engineering community, without traditional barriers of entry (e.g., cost of attendance, travel, abstract submission). In addition, over the past two years, we have developed multiple virtual symposia to help disseminate advancements towards the larger stem cell and tissue engineering communities, including a large-scale virtual “Next Generation Tissue Engineering Symposium” in September 2021, as well as multiple trainee-organized, trainee-centered symposia on “Organoids and Organs-on-a-Chip” in August 2020 and “Next Generation Tissue Engineers” in September 2021. Each virtual program, whether in the form of the weekly “Tissue Talks” seminars or the annual trainee symposia, participants were represented from over 45 countries, aided by the immense power of social media in widespread dissemination. As these programs continue, we strive to include a diverse set of speakers, both in the inclusion of research areas all over the stem cell and tissue engineering communities, as well as including leaders in biomedical engineering from traditionally underrepresented minorities in STEM. Our groups are continuing to develop new tools to allow for dissemination of stem cell topics, accessible online and in lay-terms for

K-12 students, to help inspire future generations of bioengineers. Virtual programming during the COVID-19 era have allowed for unique communication of research and community development in a time where in-person meetings are challenging.

Funding Source: NIH P41 EB027062, NSF DGE1644869
Keywords: Outreach, Tissue Engineering, Symposium

TOPIC: IMMUNE SYSTEM

509

CONSISTENT GENERATION OF MICROGLIA FROM HIPSC USING OPTI-OX TECHNOLOGY

Fairbairn, Camilla J - *Research and Development, bit.bio LTD, Cambridge, UK*
Yates, Euan - *Research and Development, bit.bio, Cambridge, UK*
Barton, Philippa - *Research and Development, bit.bio, Cambridge, UK*
Hickman, Richard - *Research and Development, bit.bio, Cambridge, UK*
Parac, Petra - *Research and Development, bit.bio, Cambridge, UK*
Klapholz, Benj - *Research and Development, bit.bio, Cambridge, UK*
Milde, Stefan - *Research and Development, bit.bio, Cambridge, UK*
Turner, Lorinda - *Research and Development, bit.bio, Cambridge, UK*
Knights, Andrew - *Research and Development, bit.bio, Cambridge, UK*
Winfield, Victoria - *Research and Development, bit.bio, Cambridge, UK*
O'Reilly, Rose - *Research and Development, bit.bio, Cambridge, UK*
Garnett, Hannah - *Research and Development, bit.bio, Cambridge, UK*
Belli-Valleta, Germano - *Research and Development, bit.bio, Cambridge, UK*
Aruthan, Chris - *Research and Development, bit.bio, Cambridge, UK*
Galimberti, Elena - *Research and Development, bit.bio, Cambridge, UK*
Ugurlu, Ceyda - *Research and Development, bit.bio, Cambridge, UK*
Byrne, Meg - *Research and Development, bit.bio, Cambridge, UK*
Jawad, Zahra - *Research and Development, bit.bio, Cambridge, UK*
Firth, Karl - *Research and Development, bit.bio, Cambridge, UK*
Hopkins, Richard - *Translational Medicine, bit.bio, Singapore, Singapore*
Patell-Socha, Farah - *Business and Commercial team, bit.bio, Cambridge, UK*
Moreau, Thomas - *Research and Development, bit.bio, Cambridge, UK*
Bernard, Will - *Research and Development, bit.bio, Cambridge, UK*
Kotter, Mark - *CEO, bit.bio, Cambridge, UK*

Abstract: Microglia are the tissue resident macrophages of the brain, accounting for 75-80% of leukocytes and 10-15% of total cells within the central nervous system (CNS). They survey neuronal function, play roles in neurogenesis, synaptic remodeling and are the first responders to infection, thereby implicated in various CNS diseases. Currently the life sciences sector relies predominantly on rodent models to recapitulate disease states for drug discovery. However, animal models do not always recapitulate human cell and disease phenotypes. To bridge this translational gap, several in vitro human models have been developed for the study of microglia, most typically primary microglia extracted directly from either embryonic, neonatal and adult tissue. However, primary cells are limited in supply, difficult to source, and typically show donor-to-donor and user variability. There is a pressing need for functional, consistent, scalable disease-relevant human models for research on microglia and the development of therapies. We have developed a proprietary gene-targeting strategy, optimised inducible overexpression (opti-ox), that enables highly controlled expression of transcription factors to rapidly reprogram human iPSCs (hiPSCs) into somatic cell types, in a scalable manner. To this end, we have generated microglia, termed ioMicroglia, using opti-ox precision reprogramming. Through transcription factor-driven reprogramming using opti-ox™ technology, hiPSCs are converted, within days, to functional microglia. ioMicroglia, 10 days post-revival, display typical morphology and express key phenotypic markers (TMEM119, P2RY12, IBA1, CX3CR1, CD11b, CD45, and CD14). RNA sequencing demonstrates that ioMicroglia have a transcriptomic signature similar to primary adult and foetal microglia. Functionally, ioMicroglia phagocytose Amyloid Beta and bacterial particles, secreting the proinflammatory cytokines IL-6 and TNFα upon stimulation. In addition, ioMicroglia can be co-cultured with glutamatergic (ioGlutamatergic) neurons and maintain their function. Using gene engineering and precision cell reprogramming, we have developed ioMicroglia, providing a functional, scalable, easy-to-use hiPSC-based model system for basic research and drug discovery applications in the CNS and immune space.

Keywords: Microglia, Precision reprogramming of hiPSCs, opti-ox

TOPIC: KIDNEY

511

LOGICAL MODELLING OF GENE REGULATORY NETWORK IN KIDNEY ORGANOIDS

Nim, Hieu T. - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Lawlor, Kynan - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Little, Melissa - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Ramialison, Mirana - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*

Abstract: Kidney organoids generated from human pluripotent stem cells (hPSC) are powerful tools in studying kidney development and diseases, but the underlying gene regulatory networks are poorly mapped. By employing recent advances in literature mining and network modelling techniques, we could now perform a systematic investigation of the complex gene regulatory network involved in kidney organoid formation. We present a gene regulatory network of the key transcription factors underlying kidney organoid formation, by mining the literature of kidney organoids and kidney development. Using Boolean network modelling language, we simulated the model to explore the transition of the kidney organoid across the intermediate mesoderm, nephron progenitors and nephron stages. Our network model revealed the intricate web of connectivity, with positive and negative feedback loops that influence the organoid formation process. Model simulations recapitulated the biological states observed during the kidney organoid formation process. Using attractor analyses,

the Boolean network model further mimics the temporal expression patterns across different stages during organoid formation. Kidney organoids are influenced by complex genetic circuitries, and systems-level investigation of the underlying gene regulatory network could provide a key to explaining the heterogeneity observed during the organoid formation process. Our model provides such an opportunity to study and engineer the networks required to generate kidney organoids in a robust and reproducible manner.

Keywords: kidney organoid, gene regulatory network, Boolean network

TOPIC: NEURAL

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SPATIALLY CONTROLLED CONSTRUCTION OF MULTI-ORGANOID NEURAL TISSUES USING BIOPRINTING

Roth, Julien G. - *Stem Cell Biology and Regenerative Medicine, Stanford University, Los Altos, CA, USA*
Shin, Sungchul - *Materials Science and Engineering, Stanford University, Stanford, CA, USA*
Brunel, Lucia - *Chemical Engineering, Stanford University, Stanford, CA, USA*
Huang, Michelle - *Chemical Engineering, Stanford University, Stanford, CA, USA*
Liu, Yueming - *Materials Science and Engineering, Stanford University, Stanford, CA, USA*
Pasca, Sergiu - *Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA*
Heilshorn, Sarah - *Materials Science and Engineering, Stanford University, Stanford, CA, USA*

Abstract: The development of the nervous system is predicated upon spatiotemporally controlled interactions between cells from distinct lineages. Human neural assembloids, 3D tissues derived from the integration of brain region-specific organoids, have mediated investigations into key developmental processes. While assembloids enable temporal control of the interactions between organoids, spatial control of their fusion would improve the in vitro recapitulation of complex in vivo physiologies including cell migration and synapse formation. Bioprinting has recently been adapted to create precisely arranged osteogenic and cardiac tissue from spheroids. However, we demonstrate here that these aspiration-mediated approaches are poorly suited for the fabrication of neural assembloids as neural organoids exhibit large diameters and relatively weak surface tension. We therefore developed a platform consisting of an iron-oxide nanoparticle laden hydrogel and magnetized 3D printer to mediate the fusion of human induced pluripotent stem cell (iPSC) derived neural organoids. We identify cellulose nanofibers (CNFs) as an ideal medium for encasing organoids with magnetic nanoparticles as they are amenable to bioorthogonal, on-demand degradation. Mixing CNFs with Carbopol creates a shear-thinning, self-healing support hydrogel that prevents organoid desiccation and stabilizes placement in 3D. We leverage this system to create precisely arranged assembloids with high cell viability and maintenance of the internal cytoarchitecture of individual organoids. Fusion occurs over four days post-printing and the resultant assembloid undergoes neural maturation over time. Finally, within custom-designed 3D reservoirs fabricated with stereolithography 3D printing, we demonstrate the differentiation, printing, and assembly of dorsal and ventral iPSC-derived forebrain organoids in a fully enclosed microenvironment. In conclusion, this platform represents a significant advance in the emulation of large, compositionally diverse tissues and enables high-throughput studies of neural development and disease.

Keywords: Neural Assembloid, Bioprinting, Hydrogel

TOPIC: NT - GENERAL

515

THERAPEUTIC EFFECTS OF POLYDEOXYRIBONUCLEOTIDE IN AN IN VITRO NEURONAL MODEL OF ISCHEMIA/REPERFUSION INJURY

Baek, Dawoon - *Department and Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Seoul*
Jo, Seongmoon - *Department of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*
Cho, Yonhee - *Department of Medicine, Yonsei University College of Medicine, Seoul, Korea*
Kim, Sung Hoon - *Department of Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Baek, Ahreum - *Department of Rehabilitation Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea*
Cho, Sung-rae - *Department of Rehabilitation Medicine, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Polydeoxyribonucleotide (PDRN) is known as an agonist that selectively stimulates adenosine A2a receptor which has a suppressing effect on the production of pro-inflammatory cytokines. Ischemia/reperfusion (I/R) injury plays a major role in the pathogenesis of ischemic stroke by provoking neuroinflammation. Thus, this study aimed to investigate the therapeutic effects of PDRN in a cell model of I/R injury. Neuro-2a was differentiated with retinoic acid and established for the cell model of I/R injury under a subsequent 3-hour exposure to oxygen and glucose deprivation (OGD) condition. Following OGD, 100 μg/ml PDRN was treated to the injured cells for 18 hours under reoxygenation condition. Transcriptome analysis was performed to investigate a potential mechanism of PDRN treatment. Differentially expressed genes related to inflammatory response and JAK-STAT pathway such as CSF1, IL-6, PTPN6, RAC2 and STAT1 were analyzed using the Database of Annotation Visualization and Integrated Discovery program, which yielded a Kyoto Encyclopedia of Genes and Genomes pathway. Real-time quantitative polymerase chain reaction, western blot, and terminal deoxynucleotidyl transferase dUTP nick end labeling assay were conducted to validate the effects of PDRN treatment. PDRN significantly reversed the expression levels of genes related to inflammatory response and JAK-STAT pathway in I/R injury-induced neuronal cells. In addition, PDRN could reverse the decreased levels of adenosine A2a receptor expression in I/R injury-induced neuronal cells. Moreover, PDRN significantly inhibited the apoptotic process in I/R injury-induced neuronal cells. Taken together, our data suggested that PDRN alleviates inflammatory response through adenosine A2a receptor mediated inhibition of JAK-STAT pathway with downregulation of neuronal death in I/R injury-induced neuronal cells. These results provide insight into potential therapeutic approaches involving PDRN treatment applied to other neurological diseases.

Funding Source: This research was funded by the NRF-2022R1A2C1006374, the KHIDI (HI21C1314), and (KFRM)

(21A0202L1 and 21C0715L1).
Keywords: Polydeoxyribonucleotide, Ischemia/reperfusion (I/R) injury, Therapeutic effects

519

REAL-TIME OXYGEN SENSING FOR CHARACTERIZATION OF STEM CELL CULTURE ENVIRONMENTS OVER TIME

Phang, Katelynn - *Research and Development/Internal Applications, Lucid Scientific, Inc., Atlanta, GA, USA*
Bryan, Richard - *Software and Data Analysis, Lucid Scientific, Inc., Atlanta, GA, USA*
Marvets, Tanner - *Research and Development, Lucid Scientific, Inc., Atlanta, GA, USA*

Abstract: Numerous factors have been shown to affect stem cell growth and maintenance of stemness, but little is known about the effects of oxygen concentration in media on stem cell proliferation and differentiation. It has long been assumed that typical ambient air oxygen concentration is sufficient for stem cell growth, but recent discoveries have highlighted the importance of in media and environmental oxygen concentration levels reflecting in vivo conditions as closely as possible. These advances in stem cell culture and differentiation are leading to changes in the setup of in vitro stem cell culture experiments that require better control and characterization of the oxygen environment in the media around the cells. This environment is a function of both the atmospheric oxygen concentration as well the changes in oxygen concentration caused by cellular consumption. To address the aforementioned need, we have developed a real-time, oxygen sensing platform for measuring both oxygen concentration and oxygen consumption rates (OCR) of many cell types (including iPSCs) in standard multiwell plates. Here, we describe the operating principles and usage of the system as well as present oxygen data measured from cell culture experiments showing cell growth over time. These experiments show the stark differences in oxygen consumption and oxygen concentration between different seeding densities of C2C12 cells through the culture period. For low seeding densities (500, 750, 1000, 1250, and 1500 cells/well), OCR stabilizes at 50-60 fmols/mm2/s after 65-90 hours of culturing and the cells are observed to be partially differentiated. Initial OCR differences between the low densities are read to be 1 fmols/mm2/s per 250 cell difference. OCR rises to and then plateaus at 120 -150 fmols/mm2/s around 30 hours after a media change as the cells become fully differentiated. For higher densities (5k, 10k, 20k, and 40k cells/well), OCR also stabilizes at the same level (50-60 fmols/mm2), but this stabilization occurs much faster (around 30 hours for 40k cells/well). These results demonstrate the value of the use of real-time oxygen sensing for better characterization and understanding of cell cultures, including stem cell cultures, and differentiation over time.

Keywords: oxygen, real-time, differentiation

521

MULTIGENE KNOCKOUT AND MULTIMODAL GENE REGULATION WITHOUT DOUBLE-STRANDED BREAKS USING DERIVED CRISPR-CAS SYSTEMS

Haupt, Amanda - *Research and Development, Horizon Discovery Ltd, Lafayette, CO, USA*
Blassberg, Robert - *Research and Development, Horizon Discovery, Cambridge, UK*
Hemphill, Kevin - *Research and Development, Horizon Discovery, Lafayette, CO, USA*
Mills, Clarence - *Research and Development, Horizon Discovery, Lafayette, CO, USA*
Rushton, Michael - *Research and Development, Horizon Discovery, Cambridge, UK*
Strezoska, Zaklina - *Research and Development, Horizon Discovery, Lafayette, CO, USA*
van Brabant Smith, Anja - *Research and Development, Horizon Discovery, Lafayette, CO, USA*

Abstract: CRISPR-based knockout (CRISPRko) and interference (CRISPRi) have been developed as powerful tools for the study of cell health and disease. While CRISPRi has a distinct set of advantages, orthogonal methods for gene perturbation such as RNA interference (RNAi) can bring additional levels of confidence to a study. Similarly, CRISPR knockout with wildtype Cas9 is increasingly popular, but alternate methods such as base editing, where a double strand DNA break is avoided, can provide result validation through a unique knockout pathway. Here, we demonstrate optimized methods for delivery of non-integrating RNAi and CRISPRi reagents into human iPSCs for phenotypic read-out at time points of less than 96 hours. Additionally, we demonstrate multiplex gene knockout with Horizon's modular Pin-point™ base editing (PnP BE) system, that uses a Cas9 nickase with an aptameric guide RNA to recruit a deaminase to the site of interest, facilitating highly efficient and precise nucleotide conversion. Compared to wildtype Cas9 gene knockout, the PnP BE system allows for simultaneous knockout of multiple gene targets in iPSCs with significantly higher viability and without affecting differentiation potential. For more complex multiplex perturbation, we demonstrate proof of concept simultaneous gene knockout and activation of two different genes in the same cell population with a wild type Cas9-VPR mRNA that can utilize 14-mer synthetic sgRNAs to initiate CRISPR activation (CRISPRa) co-delivered with 20-mer synthetic sgRNAs to initiate gene knockout. Initial experiments resulted in approximately 10,000X activation of TTN and over 20% knockout of PPIB as assessed by RT-qPCR and T7E1 mismatch detection assay, respectively. Together, these tools open new possibilities for multimodal gene regulation of multiple gene targets.

Keywords: knockdown, Base editing, Gene editing

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NOVEL 3D HYDROGEL SYSTEM ENHANCES REGENERATIVE CAPACITY OF STEM CELLS AND IMPROVES TAILORABILITY OF BIOLOGICS

Hodge, Jacob G - *Bioengineering, University of Kansas, Kansas City, KS, USA*
Robinson, Jennifer - *Chemical & Petroleum Engineering, University of Kansas, Lawrence, KS, USA*

Mellott, Adam - *Plastic Surgery, University of Kansas Medical Center, Kansas City, KS, USA*

Abstract: Traditional 2D culture plastic is not ideal for expansion of cells, particularly “stem-like” cells such as Mesenchymal Stem Cells (MSCs), and can result in a loss of multipotency, induction of senescence, and decreased bioactivity. MSC-derived therapies are promising, but the utilization of standard 2D culture techniques continues to be a significant limitation to overall therapeutic efficacy. We set out to develop a modular 3D system with tailorable properties. In so doing, we aimed to generate a system that improves MSC viability and “stem-like” phenotypic characteristics overtime in culture, subsequently resulting in more consistent and effective therapeutic products. In these studies, human MSCs were expanded in either traditional 2D culture or our novel 3D system. The 3D system is a customizable bioprinted hydrogel formulated to mechanically mimic the native soft tissue of origin for specific cell populations, including both bioinert and bioactively modified formulations. The 3D system contains a unique microarchitectural design that promotes cell migration, proliferation, and eliminates the need to subculture cells. Culture of MSCs within the bioinert 3D system provided a means to directly observe the effect of 3D culture and substrate mechanics on MSC functionality, without the use of any bioactive modulators. Our data demonstrated a significant reduction in senescence and increased expression of “stem-like” markers in MSC populations over time within the bioinert 3D system. Proteomic evaluation of the Conditioned Media (CM) indicated that 3D expansion significantly increased the secretion of regenerative factors, including the EGF, HGF, IGF, and FGF protein families. Similarly, co-culture of a secondary cell population with CM demonstrated significant enhancement of cell proliferation, migration, and metabolic activity when treated with MSC-CM from 3D relative to 2D. Subsequently, culture of MSCs within the bioactively modified 3D system resulted in MSC-CM that further augmented the proliferation, migration, and metabolic activity of the secondary cell population, relative to the bioinert 3D system. Together, this data demonstrates the potential of a novel 3D system to enhance the overall regenerative capacity of MSCs over time, with the added capability to tailor MSC-derived biologics.

Keywords: 3D Hydrogel System, Stem Cell Secretome, Cell Culture Expansion

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IT TAKES A VILLAGE: A NOVEL SYSTEM FOR LARGE-SCALE HPSC CULTURE

Neavin, Drew - *Cellular Science, Garvan Institute of Medical Research, Darlinghurst, Australia*
Steinmann, Angela - *Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Daniszewski, Maciej - *Anatomy and Physiology, The University of Melbourne, Melbourne, Australia*
Chiu, Han - *School of Biomedical Sciences, Institute for Molecular Bioscience, Brisbane, Australia*
Moutinho, Cátia - *Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Chan, Chia-Ling - *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Tyebally, Mubarika - *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Gnanasambandapillai, Vikkitharan - *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Lam, Chuan - *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Nguyen, Uyen - *Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*
Hernández, Damián - *Anatomy and Physiology, The University of Melbourne, Melbourne, Australia*
Lidgerwood, Grace - *Anatomy and Physiology, The University of Melbourne, Australia*
Hewitt, Alex - *School of Medicine, Menzies Institute for Medical Research, Hobart, Australia*
Pebay, Alice - *Anatomy and Physiology, The University of Melbourne, Australia*
Palpant, Nathan - *School of Biomedical Sciences, Institute for Molecular Bioscience, Brisbane, Australia*
Powell, Joseph - *Cellular Genomics, Garvan Institute of Medical Research, Darlinghurst, Australia*

Abstract: The large-scale culture of human pluripotent stem cells (hPSCs) (100s - 1000s of lines) has been historically challenging due to prohibitive costs and handling time required. Further, culturing hPSC lines in different plates and batches creates technical artifacts. This has restricted the ability to address biological questions that require experimental designs of hundreds of genetically diverse lines. For example, investigating the relationship between genetic variation and cellular phenotypes via single-cell expression quantitative trait locus (eQTL) studies require large-scale culture. Recently, we and others have developed ‘village in a dish’ approaches to solve these limitations by drastically increasing the scale of hPSC studies though co-culture and differentiation of multiple hPSC lines in a single dish. We employ single-cell RNA-seq (scRNA-seq) to assign cells to the correct hPSC line using the inherent genetic profiles captured in the transcriptome of each cell. We set out to evaluate whether hPSCs cultured in the same dish alters the transcriptional profiles of companion lines. To address this, we designed a series of experiments to interrogate the transcriptional profiles of hPSCs cultured independently (uni-culture) and in villages and replicated these experiments across three independent laboratories. Using linear mixed models, we show that the transcriptional profiles of lines cultured independently and in villages are highly concordant ($0.46 < R < 0.88$). The observed transcriptional variation was predominantly a result of donor effects, with limited evidence of variation due to the village culture system. Even when village effects were detected, line-specific effects were still consistently observed ($0.82 < R < 0.94$) – indicating that any potential influence of a village system does not alter line-specific effects. These results replicate across all three laboratory sites, leading us to conclude that the transcriptional profiles and landscape of cell states is consistent between uni- and village-culture systems. Collectively, our results show that our village method can be effectively used to detect hPSC line-specific effects, including sensitive dynamics of cell states.

Keywords: Population genomics, Single-cell RNA-seq, Stem Cells

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FUNCTIONALLY TAILORED BIOMIMETIC MATRICES FOR CHEMICALLY DEFINED CULTURE OF STEM CELLS AND THEIR DERIVATIVES

Segeletz, Sandra - *Innovation and Research, denovoMATRIX GmbH, Dresden, Germany*
Thamm, Kristina - *Project Services, denovoMATRIX, Dresden, Germany*
Patino, Michelle - *Innovation and Reserach, denovoMATRIX, Dresden, Germany*
Wetzel, Richard - *denovoMATRIX, Dresden, Germany*
Wobus, Manja - *Medizinische Klinik und Poliklinik I, 2University Hospital Carl Gustav Carus der Technischen Universität Dresden, Dresden, Germany*

Abstract: Stem cells are resourced for cell therapies due to their ability to self-renew and differentiate into specialized cell types. To exploit their full potential, we must consider their microenvironment, an organized combination of extracellular matrix (ECM), cells, and interstitial fluid that regulates cell fates. The ECM is composed of glycosaminoglycans (GAGs) and structural proteins with binding sites for specific integrins. For cell therapies, the culture of anchorage-dependent cells in vitro is facilitated by protein-, peptide-, and polymer-based substrates. However, the simple composition neither reflects the in vivo niche nor considers potentially different needs of stem cells during ex vivo culture processes and thereby may limit their therapeutic potential. To functionally recreate the plethora of signals presented by the ECM, yet simplifying it to a minimal system, we develop biomatrices that combine synthetic biofunctional peptides with GAG mimetics, modular in composition and concentration. Using this system, we created a library of 96 different microenvironments to screen for biologically relevant compositions for ex vivo cell expansion, propagation, and differentiation. Using this system we identified process- and cell-specific biomatrices that promote long-term expansion of mesenchymal stromal cells (MSCs) and induced pluripotent stem cells (iPSCs), facilitating MSCs isolation in serum-/xeno-free conditions, and differentiation into the three germ layers. Each of these biomatrices has a unique design that improves cell proliferation, isolation efficiency, maintenance of stemness, and/or differentiation capacity. Interestingly, the biomatrices for differentiation of iPSCs into mesoderm, MSC isolation, and MSC proliferation are distinct in terms of composition and component concentration. This illustrates that the same cell type prefers distinct microenvironments during different stages of in vitro culture. To support scale-up processes, we applied the coating onto synthetic and natural 3D scaffolds and developed ready-to-use microcarriers for the culture of MSCs and iPSCs. Thus, our modular, chemically defined and scalable technology enables the development of high-performance and reproducible cell culture protocols for research and cell therapy applications.

Funding Source: denovoMATRIX GmbH was supported and received funding by the European Social Fund (ESF), the European Regional Development Fund (ERDF), and by the EXIST-Forschungstransfer granted by the BMWi.
Keywords: biomatrices, mesenchymal stromal cells, 3D culture

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XENO-FREE AND CHEMICALLY DEFINED CULTURE OF HUMAN PLURIPOTENT STEM CELLS ON A SYNTHETIC SUBSTRATE

Perez-Chavez, Agustin E. - *Bioengineering, Oakland University, Troy, MI, USA*
Madlambayan, Gerarld - *Biological Sciences, Oakland University, Rochester, MI, USA*
Timilsina, Suraj - *Biological Sciences, Oakland University, Rochester, MI, USA*

Abstract: The fields of tissue engineering, regenerative medicine, and developmental biology are being revolutionized by the use of embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) of human origin. This potential will be maximized by an improvement in culture conditions that provide reproducible, xeno-free, and defined conditions. While the development of several chemical-defined and xeno-free culture mediums has been successful, their use with undefined animal-derived extracellular matrix substrates (Matrigel for example), setback their benefits. Previously, we demonstrated the long-term culture of hPSCs on PMEDSAH-grafted plates with a xeno-free human-cell conditioned medium. Here, we describe a systematic study to identify optimal conditions for the use of our synthetic substrate with multiple chemically-defined and xeno-free medium formulations. Pre-conditioning of our synthetic substrate with “an adapting solution” resulted in long-term culture (20 passages) of hPSCs with xeno-free and chemically defined medium. This also resulted in a significant enhancement in the expansion of pluripotent cells without chromosomal abnormalities. This culture condition also supported a higher reprogramming efficiency of somatic cells into iPSCs compared to non-defined culture conditions. In conclusion, we optimized the culture and derivation of hPSCs in conditions that might be used in obtaining large populations of cells required for many applications in regenerative and translational medicine.

Funding Source: This research was supported with funding from the Oakland University REF funding, 9 and the NSF grant 2026049.
Keywords: human pluripotent stem cells, xeno-free culture, synthetic materials

531

SUSTAINED FGF2 DELIVERY USING A BIOENGINEERED DISC DEVICE IMPROVES HUMAN STEM CELL PLURIPOTENCY, REDUCES SPONTANEOUS DIFFERENTIATION, AND IMPROVES DIRECTED DIFFERENTIATION.

Bertucci, Taylor - *Neural Stem Cell Institute, Rensselaer, NY, USA*
Lotz, Steve - *Neuracell, Neural Stem Cell Institute, Rensselaer, NY, USA*
Borden, Susan - *Neuracell, Neural Stem Cell Institute, Rensselaer, NY, USA*
Bull, Victoria - *Stem Cultures LLC, Rensselaer, NY, USA*
Stevens, Katie - *Neuracell, Neural Stem Cell Institute, Rensselaer, NY, USA*
Lane, Keith - *Neuracell, Neural Stem Cell Institute, Rensselaer, NY, USA*
Goderie, Susan - *Neuracell, Neural Stem Cell Institute,*

Rensselaer, NY, USA
Chowdhury, Rebecca - *Neural Stem Cell Institute, Rensselaer, NY, USA*
Joy, Shona - *Neural Stem Cell Institute, Rensselaer, NY, USA*
Leslie, Erin - *Stem Cultures LLC, Rensselaer, NY, USA*
Lotz, Ryan - *Neuracell, Neural Stem Cell Institute, Rensselaer, NY, USA*
Roberts, Kristina - *Stem Cultures LLC, Rensselaer, NY, USA*
Arduini, Brigitte - *Neural Stem Cell Institute, Rensselaer, NY, USA*
Stern, Jeffrey - *Stem Cultures LLC, Rensselaer, NY, USA*
Temple, Sally - *Neural Stem Cell Institute, Rensselaer, NY, USA*

Abstract: High-quality human pluripotent stem cells (PSCs) cultures are imperative for high-quality downstream applications. FGF2 is a required signal for maintaining PSCs in an undifferentiated state. FGF2 has a short half-life and degrades within hours in culture medium. PSC cultures require frequent, often daily, medium changes to replenish FGF2. This results in large fluctuations in FGF2 levels and removal of conditioning factors, which can increase cell stress. With daily feeding, PSC lines often recover poorly from thaw and show spontaneous differentiation that necessitates laborious clean-up. We have developed FGF2 DISCs, in which control-release FGF2 microbeads are encased within a non-degradable hydrogel. The FGF2 DISC enables easy addition and removal from cultures. A single FGF2 DISC provides a stable 10 ng/ml level of native FGF2 over a 7-day culture period. Use of FGF2 DISCs reduces PSC feeding schedule from daily medium changes to 1 or 2 medium changes weekly. By minimizing swings in FGF2 levels, the new FGF2 DISC method shows consistent improvement across all cell lines and PSC-maintenance media backgrounds that we have tested. PSC cultures grown with DISCs recover quicker from a thaw, and with less spontaneous differentiation. This reduces the need for culture clean-up and allows faster transition to differentiation experiments. When grown with DISCs, PSCs exhibit increased pluripotency gene expression and reduced expression for genes diagnostic of spontaneous differentiation. Following maintenance in multiple PSC media backgrounds, PSCs grown with FGF2 DISCs showed enhanced efficiency of trilineage differentiation. More homogeneous PSC cultures grown with DISCS produced consistent 3D cerebral organoids composed of more homogeneous neural progeny than standard PSC protocols, a result observed across multiple PSC lines. Hence, FGF2 DISCs achieved improved consistency in PSC culture with improved directed differentiation efficiency while reducing medium changes. The FGF2 DISC technology is compatible with multiple media and culture formats.

Funding Source: NIH STTR
Keywords: pluripotent stem cell media, controlled-release growth factor, brain organoid culture

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GENERATION OF ENGINEERED PLURIPOTENT STEM CELL LINES FOR CLINICAL APPLICATIONS

Pierpoint, James - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Peralta, Dulce - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Andronikou, Nektaria - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Sangenario, Lauren - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Kuninger, David - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Potter, Jason - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Pochanard, Pandhita - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Kovarcik, Don Paul - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*
Willems, Erik - *Cell Biology, Thermo Fisher Scientific, Carlsbad, CA, USA*

Abstract: Human induced pluripotent stem cells (hiPSCs) have been globally recognized as a multipurpose research tool for modeling human disease and biology, screening and developing therapeutic drugs, and implementing cell and gene therapies. The ability to differentiate human iPSCs into any cell type supports the study of biology and disease in these specified cells in vitro as well as the generation of cell replacement therapies. The emergence of genome editing tools, including the CRISPR/Cas9 system or TALENs, enable genetic modification of these cells; such as introduction of single base changes or inserting reporters, bio-sensors or transgenes, which can be used to study the effects of genetic differences or to alter the biological functions of the desired differentiated cell type for allogeneic cell therapy applications. While genome engineering of hiPSC has become relatively straightforward, reagents and workflows for cGMP generation of genome edited hiPSC are currently scarce. Using the generation of immune evasive (allogeneic) hiPSC through genome engineering, we showcase a workflow that implements next generation reagents and instruments that enable cGMP manufacturing of engineered hiPSC. We demonstrate that through the use of novel GMP grade reagents, genome editing in hiPSC is highly efficient, and survival and genomic stability of edited cells through the genome editing workflow is maintained throughout the workflow. We furthermore demonstrate that available closed systems perfectly integrate into this workflow. In summary, we detail advances with tools, reagents and protocols that facilitate the genome editing workflow in hiPSC and demonstrate that the use of such tools can be readily implemented in generation of allogeneic hiPSC for clinical application, with potential applications in manufacturing of allogeneic cell therapy products.

Keywords: allogeneic PSC, clinical application, genome editing

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ECLIPSE, AN AUTOMATED CRISPR PLATFORM FOR THE LARGE-SCALE GENERATION OF CELL MODELS FOR THE IPSC NEURODEGENERATIVE DISEASE INITIATIVE (INDI)

Deng, Peter - *Product Management, Synthego, Redwood City, CA, USA*
Chair, Antinea - *MSAT, Synthego, Redwood City, CA, USA*
Holden, Kevin - *Platform Research, Synthego, Redwood City, CA, USA*
King, Anna - *MSAT, Synthego, Redwood City, CA, USA*
Morell, Montse - *Platform Research, Synthego, Redwood City, CA, USA*
Saavedra, Brandon - *Platform Research, Synthego, Redwood City, CA, USA*
Sailor, Dana - *MSAT, Synthego, Redwood City, CA, USA*
Sommer, Andreia Gianotti - *ECLIPSE Production, Synthego, Redwood City, CA, USA*

Abstract: The National Institutes of Health led iPSC Neurodegenerative Disease Initiative (iNDI) is the largest iPSC genome engineering project attempted with the goal of generating a widely

available and standardized set of diseased cell models for over 100 single nucleotide variants (SNV) mutations associated with Alzheimer's disease and related dementias (ADRD) in isogenic IPSC lines. The standardization of cell models is of vital importance for the generation of reproducible and actionable data in therapeutic development. As part of a multi-institution collaboration, Synthego was selected for the generation of 25 SNVs in the candidate KOLF2.1 iPSC line. Toward these goals, we describe the use of our automated, high throughput CRISPR editing platform, ECLIPSE, for the rapid generation of knock-in iPSC models of ADRD. We leveraged our state-of-the-art knock-in methods and automated pipelines for the design, experimental optimization, and clonal isolation of 23 of the candidate target mutations in iPSCs. For each SNV target, 3 clonal homozygous and 6 clonal heterozygous mutation lines were generated for a total of 264 clonal cell lines over a 6-month period. The utilization of automated systems such as our ECLIPSE platform are critical catalysts for the rapid development of relevant cell models in large scale disease initiatives such as iNDI.

Keywords: CRISPR, iPSC, Disease Model

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CONSTRUCTING A LIBRARY OF FLUORESCENT IPSCS VIA CRISPR/CAS9 DIRECTED GENE INTEGRATION AT AAVS1 LOCUS

Han, Chann Makara - *SCB & DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, The University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
McGrath, Patrick - *Section of Developmental Biology, Department of Pediatrics, The University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Butterfield, Kiel - *Department of Neurology, The University of Kentucky, Lexington, KY, USA*
Ferreyros, Michael - *SCB & DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, The University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Bilousova, Ganna - *SCB & DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, The University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Kogut, Igor - *SCB & DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, The University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: The isolation of human embryonic stem cells (hESCs) in 1998 ushered a new era for modeling human development and disease. However, hESCs presented issues for the research and clinical community including immune rejection, accessibility, and ethical concerns. Many of these barriers were eliminated with the development of induced pluripotent stem cells (iPSCs). Patient-specific iPSCs are a powerful tool in human disease modeling and translational research because they can be expanded indefinitely, differentiated into many cell types, and are readily genetically modified with gene editing tools such as CRISPR/Cas9. However, there are still limited tools for visualizing and tracing these cells in a complex environment. One solution is to endogenously integrate a fluorescent reporter into a safe-harbor of the iPSCs to produce a permanent and persistent expression. To accomplish this, we constructed a library of targeting plasmids that constitutively express fluorescent proteins spanning the visual spectrum from blue (405nm excitation) to far red (647nm excitation), with homology to the adeno-associated virus integration site 1 (AAVS1) for knock-in by homology directed repair using CRISPR/Cas9. Additionally, we constructed nuclear- and membrane-localized variants for each fluorescent protein (FP) for a total of 12 targeting plasmids including one FP-akaluciferase version. These plasmids were then individually targeted into an iPSC line via nucleofection, followed by clonal selection and characterization. To assess our newly generated lines, we performed trilineage differentiation, human intestinal organoid differentiation, and teratoma induction on mice. The fluorescence expression persists throughout differentiation, and localizes correctly. Our results showed that we have created a library of quality iPSC lines that have persistent and constitutive expression of the fluorescent proteins. These iPSC lines add valuable tools to the scientific community for disease modeling and other downstream applications.

Keywords: Induced Pluripotent Stem Cells (iPSCs), CRISPR/Cas9, AAVS1 safe harbor

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

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PROFILING OF SINGLE-CELL EXTRACELLULAR VESICLE SECRETION USING 3D STRUCTURED MICROPARTICLES

Koo, Doyeon - *Bioengineering, University of California, Los Angeles, CA, USA*
Udani, Shreya - *Bioengineering, UCLA, Los Angeles, CA, USA*
Di Carlo, Dino - *Bioengineering, UCLA, Los Angeles, CA, USA*

Abstract: Although extracellular vesicles (EVs) derived from Mesenchymal stem cells (MSCs) have recently shown significant potential in the treatment of disease, critical challenges remain with the study and scaling of uniform EV therapeutics for clinical translation due to the absence of methods to examine inherent heterogeneity in the secretion of EVs along with unknown mechanisms underlying their biogenesis. There is an urgent need to develop a high-throughput platform that can screen and select single-cells based on EV secretion function to enable manufacturing of EV-based therapeutics or the study of genetic underpinnings of EV biogenesis. We have developed cavity-containing hydrogel microparticles, nanovials, that can be loaded with single-MSCs. The nanovial cavity is locally functionalized with biotinylated gelatin which enables adhesion of MSCs and capture of secreted EVs through EV-specific capture antibodies conjugated to the cavity. Secreted EVs are stained with fluorescent detection antibody and the corresponding cells are sorted based on the EV production levels using commercial fluorescence activated cell sorter. We have validated that nanovials functionalized with antibodies against the MSC-EV specific marker, anti-CD63, can capture and detect purified EV samples with four orders of magnitude in dynamic range in concentration, which is sufficient for single-cell analysis. We have also demonstrated our ability to capture and label CD63+ and CD9+ secreted EVs on nanovials loaded with single-MSCs, and sorted three distinct secreting populations, including top 10% of cells with high EV secretion phenotype. Sorted single-MSCs on nanovials can also be expanded into single-cell colonies as they maintain viability and proliferate. Our approach enables screening of > 1 million cells in a day, which exceeds the throughput of previously available single-cell EV secretion assays by 1000-fold. With our approach, we can screen and isolate rare cells based on their EV secretion for direct downstream process-

es such as cell line development or molecular profiling of EVs and their cellular pathways. Genetic modifications that enhance EV production and pathways responsible for EV shedding can be further investigated. Importantly, the platform will lay improved foundation for therapeutic applications of EVs.

Funding Source: NIH R21GM142174
Keywords: Extracellular Vesicles, Single-cell Analysis, Secretory Function

TOPIC: NEURAL

857

BRAIN ORGANOIDS TO EVALUATE CELLULAR THERAPIES

Fernandez-Munoz, Beatriz - *Unidad de Producción y Reprogramación Celular (UPRC), Red Andaluza de Diseño Y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Spain*
Campos-Cuerva, Rafael - *Unidad de Producción y Reprogramación Celular (UPRC), Sevilla, Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Saint Helena*
Carlos, Casado - *Unidad de Producción y Reprogramación Celular (UPRC), Sevilla, Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Spain*
Ferrari, Daniela - *Department of Biotechnology and Biosciences, University Milan-Bicocca, Milan, Italy*
García-Delgado, Ana - *Departamento de Farmacia y Tecnología farmacéutica, Universidad de Sevilla, Spain*
Márquez-Rivas, Javier - *Departamento de Neurocirugía, Hospital Universitario Virgen del Rocío, Sevilla, Spain*
Martín-López, María - *Unidad de Producción y Reprogramación Celular (UPRC), Sevilla, Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Spain*
Rivero-Garvia, Mónica - *Departamento de Neurocirugía, Hospital Universitario Virgen del Rocío, Sevilla, Spain*
Rosell-Valle, Cristina - *Unidad de Producción y Reprogramación Celular (UPRC), Sevilla, Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Spain*
Sánchez-Pernaute, Rosario - *Unidad de Producción y Reprogramación Celular (UPRC), Sevilla, Red Andaluza de Diseño y Traslación de Terapias Avanzadas (RADyTTA), Sevilla, Spain*

Abstract: The lack of predictive models is one of the main challenges that cell therapies face. Animal models have important limitations as the underlying molecular, cellular, and physiological mechanisms are often inherently different in humans, especially in the brain. Therefore, for translation to the clinic, the development of more complex models based on human cells may be crucial. Here, we have developed an in vitro model based on human forebrain organoids to study the differentiation potential of neural stem cells (NSC), their ability to integrate into the neuronal network of the organoid, and evaluate their potential (safety and efficacy profiles) as cell therapies. We generated brain organoids from iPSC and after 1 month we identified the formation of different cerebral tissues and cell types by the expression of typical markers from ventricular zone (SOX2, NCAD), ependyma (TUBβIV, FOXJ1), chroid plexus (TTR), microglia (IBA1), oligodendrocyte precursors (OLIG2), astrocytes (GFAP) and neurons (TUJ1, DCX). After 4 months of culture, we transplanted NSC transduced with EGFP into organoids. Live imaging of NSC-EGFP injected into organoids showed that cells integrated and migrated within the human tissue. We fixed the organoids 3 weeks after transplantation and we studied the differentiation potential comparing these data with transplantation studies in the brain of NOD-SCID-γ mice. We found that NSC differentiate into neuron and oligodendrocyte precursors both in mice and human organoid models although with some differences. Our results suggest that brain organoids can be used in the evaluation of cell therapies, increasing the predictability for future treatments, since it is a human model, and could reduce the number of animals used for testing, helping the development of more effective cell therapies and being in line with the philosophy of the 3Rs.

Funding Source: This work was supported by research funds from the Fundación Alicia Koplowitz and the Instituto de Salud Carlos III- FEDER funds “Una manera de hacer Europa” through the project “DTS20/00108”.
Keywords: Neural stem cells, Cell therapy, 3Rs

TOPIC: NT - GENERAL

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SORTING AND TRANSCRIPTOMIC ANALYSIS OF SINGLE MESENCHYMAL STEM CELLS LINKED TO SECRETION USING FACS-COMPATIBLE, CAVITY CONTAINING MICROCARRIERS

Udani, Shreya - *Bioengineering, University of California, Los Angeles, CA, USA*
Koo, Doyeon - *Bioengineering, University of California, Los Angeles, CA, USA*
Baghdasarian, Sevana - *Bioengineering, University of California, Los Angeles, CA, USA*
Langerman, Justin - *Biological Chemistry, University of California, Los Angeles, CA, USA*
Cheng, Brian - *Bioengineering, University of California, Los Angeles, CA, USA*
Plath, Kathrin - *Biological Chemistry, University of California, Los Angeles, CA, USA*
Di Carlo, Dino - *Bioengineering, University of California, Los Angeles, CA, USA*

Abstract: Stem cells derive many therapeutic functions through their production of paracrine factors which modulate inflammation, promote angiogenesis, and inhibit apoptosis. Mesenchymal stem cells (MSCs), for example, are a promising treatment modality; however, disparate results in translational studies have hindered the progression of most MSC therapies past early-stage clinical trials. A barrier to translation is the inherent heterogeneity in secretory function of MSCs, which varies based on the tissue source and cell expansion conditions. There is a critical need to develop technologies to elucidate links between stem cell secretory function and regenerative potential to standardize and engineer more effective cell therapies. We present a platform for the rapid screening and sorting of individual MSCs based on secreted factors accumulated on 3D-structured microparticles using standard fluorescence activated cell sorters (FACS). Our cavity-containing hydrogel microparticles (nanovials) feature a gelatin coating on the cavity surface for single-cell adhesion and localized secreted product capture. Captured growth factors are labelled with fluorescent antibodies, and cells on nanovials are analyzed and sorted based on secretion level using the Sony Sorter SH800, achieving a dynamic range spanning 4 orders of magnitude of concentration. Unlike current platforms for cell isolation by secretion which are incompatible with adherent cells or low-throughput, such as droplet encapsulation or microwells,

nanovials provide extracellular matrix for cell anchoring and high-throughput sorting with shear stress protection. Using nanovials, we measured VEGF secretion from single adipose-derived MSCs and determined that after a 12-hour incubation period, a sub-population of ~40% of MSCs secrete VEGF above baseline levels. We demonstrated that MSCs in nanovials have much higher viability following FACS sorting than suspended MSCs (85% vs 25%), increasing recovery of functional cells. We are integrating our nanovial workflow with scRNA-seq (10X Chromium) to link the secretome with transcriptome. Overall, our nanovial platform can quantitatively screen and isolate potentially millions of viable cells by their growth factor secretion levels, which promises to improve the current paradigm of cell therapy.

Funding Source: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) R21DK128730 Broad Stem Cell Research Center (BSCRC) and California NanoSystems Institute (CNSI) Stem Cell Nano-Medicine Initiative Planning Award
Keywords: Functional Screening, Microcarriers, Single-cell Analysis

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

861

NEXT GEN BIOPROCESSING OF CELL & GENE THERAPIES

Oh, Steve W. - *Stem Cell Bioprocessing, Bioprocessing Technology Institute, Singapore, Singapore*

Abstract: As cell and gene therapies move closer to the clinic, new ways of bioprocessing these entities have to be developed. We present solutions that address the demands of allogeneic cell manufacturing such as: 1) Selection of high quality donors of mesenchymal stromal cells (MSC) prior to bioprocessing, 2) development of serum free media that perform in 2D and suspension cultures and 3) a novel Bach impeller which generates mixing at low power numbers and yet achieves high density. A key requirement for allogeneic stem cell manufacturing is selecting for donors which can yield cells that are potent and that can be passaged to high numbers of population doublings. We show evidence that GSTT1 (glutathione S-transferase T1) null donors have 3 times higher population doublings in long term in vitro cultures, compared to GSTT1 heterozygous and homozygous donors. By testing for this marker prior to harvesting bone marrow for MSC, we are able to eliminate 65% of poor donors from the pool and harvest highly potent MSC as shown by colony forming unit (CFU) efficiency and secretion of cytokines. Secondly, we have developed a novel serum free media which can enable 7 times higher population doublings at P8, compared to two commercially available serum free media and the serum control. Enhanced performance was demonstrated in 2D and microcarrier suspension cultures, MSC grown in this media have similar CFU efficiency but 50% higher chondrogenic potential as shown by the chondrogenic pellet assay. Thirdly, we present engineering characterization of a Bach impeller which has an exponential spiral design with 5 vanes for mixing cells and microcarriers in suspension bioreactor. Compared to the industrial standard of 2 blade marine and 3 blade segment impellers, the Power number of this impeller is low (0.4) vs. 0.3 and 0.75 at high Reynolds numbers. This means that mixing is equivalent, but less energy is applied which can damage cells on microcarriers at high shear rates. We also demonstrate the achievement of a high density of 1.5 M cells/ml of MSC without clumping of the microcarriers in a 1 litre batch culture over 6 days with 11.2 mg/ml of microcarriers. In summary, these three examples of bioprocess developments are generally applicable to cell and gene therapy manufacturing which can improve the quality and quantity of MSC for therapeutic applications.

Funding Source: Agency for Science Technology and Research (A*STAR)
Keywords: Stem cell bioprocessing, Cell therapy, Donor selection

POSTER SESSION II: ODD

3:00 PM – 4:00 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: CARDIAC

603

DOWNREGULATION OF CDH18 IN EPICARDIAL CELLS INDUCES EMT AND DIRECTS CELL FATE TOWARDS SMOOTH MUSCLE CELL DIFFERENTIATION

Junghof, Julia - *Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan*
Tian, Yu - *Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto, Japan*
Lucena-Cacace, Antonio - *Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto, Japan*
Yoshida, Yoshinori - *Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto, Japan*

Abstract: The epicardium, which is the outer layer of the heart wall covering the myocardium, serves as a progenitor source for different cardiac lineage descendants, thus supporting cardiac development, repair and regeneration. During cardiogenesis, epicardial cells undergo an epicardial-to-mesenchymal transition (EMT) to give rise to epicardial-derived cells (EPCDs). Finely tuned signaling regulates the cell plasticity and cell-fate decisions of EPCDs. Interestingly, the epicardium reactivates after cardiac injury, playing a major role during tissue remodeling and cardiac regeneration. However, the mechanism governing the biology of the epicardium and its repair and regeneration processes are poorly understood. Previously we identified type II classical cadherin CDH18 as a specific epicardial marker that is lost in EPDCs. Here, we show that the downregulation of CDH18 leads to the loss of epicardial identity and promotes the onset of EMT. We found this effect to be more apparent in cells representing a more pro-epicardial state compared to cells representing a fetal-like epicardial stage. The loss of CDH18 in combination with the loss of TCF21 resulted in cell-fate-specific differentiation towards smooth muscle cells (SMCs). This cell-fate specific differentiation behavior was observed even in conditions promoting cardiac fibroblast fate. SMCs that were induced by the downregulation of CDH18 behaved similarly to SMCs that were induced by cytokine treatment. Furthermore, we verified the activation of pathways related to EMT and SMC development upon silencing CDH18. Finally, we showed that the expression of GATA4 was correlative to CDH18

and that the downregulation of GATA4 resulted in an equal reduction of CDH18. Our results will aid in investigating epicardial function in human development and disease, especially EMT and cell-fate decision, processes that are particular important for cardiac repair.

Keywords: Epicardium, EMT, Cell-fate decision

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

605

INVESTIGATING TIE2 SUPERCLUSTER FOR ENDOTHELIAL TIGHT JUNCTION FORMATION TO PROMOTE VASCULAR STABILITY

Zhao, Yan Ting - *Oral Health Sciences, University of Washington, Seattle, WA, USA*
Saini, Shally - *Biochemistry, University of Washington, Seattle, NM, USA*
Ueda, George - *Biochemistry, University of Washington, Seattle, WA, USA*
Somasundaram, Logeshwaran - *Biochemistry, University of Washington, Seattle, WA, USA*
Ben-Sasson, Ariel - *Biochemistry, University of Washington, Seattle, WA, USA*
Mathieu, Julie - *Comparative Medicine, University of Washington, Seattle, WA, USA*
Sellers, Drew - *Bioengineering, University of Washington, Seattle, WA, USA*
Baker, David - *Biochemistry, University of Washington, Seattle, WA, USA*
Ruohola-Baker, Hannele - *Biochemistry, University of Washington, Seattle, WA, USA*

Abstract: The angiopoietin-Tie2 pathway regulates blood vessel stability, remodeling, and permeability. However, the mode of activation and downstream activity are not presently fully understood. Angiopoietin-1 (Ang1) and -2 (Ang2) modulate Tie2 signaling via almost identical receptor-binding domains (F-domain). However, while Ang1 promotes activation of downstream pAKT, Ang2 can antagonize this Ang1 dependent effect. We utilized computationally designed protein scaffolds conjugated with the Ang1 F-domain at a range of valencies (3, 4, 6, 8, 12, 30, and 60) and geometries to probe the molecular basis of specific Tie2 downstream signaling output and the subsequent molecular events leading to tight junction formation. We found 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. Moreover, recently we have shown that F-domains conjugated to two-dimensional protein arrays can generate Tie2 superclusters. Our preliminary data suggest that Tie2 superclusters recruit Integrin, VE-Cadherin, PECAM1, and ZO1 suggestive of tight junction initiation. We also observe Tie2 agonistic F-domain scaffolds accelerate ZO1 re-assembly after chemical disruptions. We will now study the requirement of these components in the Tie2 pathway using loss-of-function, computer-designed protein, and structural analysis. When examined in vivo, super-agonist icosahedral nanoparticles displaying 60 F-domains caused significant revascularization in hemorrhagic brains after a controlled cortical impact injury. These Tie2 super-agonists may in the future have therapeutic applications in tissue regeneration, wound healing, and cancer therapy. Our computational design approach to investigate the role of ligand valency and receptor engagement is broadly applicable to a variety of signaling pathways.

Funding Source: NIH/NIDCR #T90 DE021984 and NIH TL1 TR002318 for YTZ
Keywords: tight junctions, Tie2, integrin

TOPIC: EPITHELIAL_GUT

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L1 CELL ADHESION MOLECULE MAINTAINS TISSUE HOMEOSTASIS BY REGULATING INTESTINAL STEM AND PROGENITOR CELL PROLIFERATION

Jackson, Nicholas - *Molecular Biology Institue, University of California, Los Angeles, San Francisco, CA, USA*
Jones, Leanne - *Anatomy Department, University of California, San Francisco, CA, USA*

Abstract: Intestinal stem cells (ISCs) are a specialized, multipotent population of adult stem cells responsible for maintaining intestinal homeostasis. ISCs reside in a tightly regulated and protective stem cell niche at the base of the crypt and are controlled by intrinsic, extrinsic, and systemic cues. The L1 cell adhesion molecule (L1CAM) is a transmembrane glycoprotein and a member of the immunoglobulin superfamily of cell adhesion molecules. Although L1CAM is best characterized in the development of the nervous system, expression in other tissues, including the murine small intestine (SI), has been described. By contrast, a recent study suggested that L1CAM is not expressed in healthy human intestinal tissue but, rather, it is induced during cancer progression and important for driving colon cancer metastasis. Our goal is to elucidate the role of L1CAM in healthy intestinal tissue. We characterized the pattern of L1CAM expression in the murine and human intestine and test a role for L1CAM in regulating the behavior of ISCs. In the murine SI, L1CAM is expressed in proliferative cells in the transit amplifying (TA) zone, whereas in the colon, L1CAM is expressed in rare cells at the base of the crypt. In sections from human intestine, L1CAM is expressed in rare cells at the base of the crypt in the SI and the colon. In addition, L1CAM is expressed in 3D intestinal organoids generated from human and mouse SI and colon crypts. Blocking L1CAM function in organoids derived from mouse colon, using an antibody that is specific to the extracellular domain, resulted in a suppression of organoid growth and proliferation. Therefore, we conclude that L1CAM is expressed in normal intestinal tissue in both mouse and humans and that it plays an important role in regulating ISC and progenitor cell proliferation. Given that L1CAM expression is upregulated in both inflammatory bowel disease and colorectal cancer, our findings provide an important framework for determining the role/s of L1CAM in maintaining homeostasis of normal intestinal tissue. Furthermore, our future work will untangle discrepancies in the role L1CAM plays in colorectal cancer initiation.

Funding Source: UCLA Broad Stem Cell Research Center UCLA Digestive Diseases Research Center UCLA Cellular and Molecular Biology Training Program Jonsson Comprehensive Cancer Center
Keywords: Intestine, Organoid, L1CAM

TOPIC: EPITHELIAL_SKIN

611

NICHE ARCHITECTURE DICTATES STEM CELL LOCATION AND FUNCTION IN THE HAIR FOLLICLE

Wei, Haoyang - *Genetics, Yale University, New Haven, CT, USA*
Xin, Tianchi - *Genetics, Yale University, New Haven, CT, USA*
Greco, Valentina - *Genetics, Cell Biology and Dermatology, Yale Stem Cell Center, Yale Cancer Center, Yale University, New Haven, CT, USA*

Abstract: Stem cells and their nearby niche cells generate an architecture essential for organ function. During hair growth, the hair follicle remodels its epithelial architecture through stem cell behaviors under the instruction of a juxtaposed fibroblast niche -- dermal papilla. Although dermal papilla's signaling crosstalk with hair follicle epithelium is well-characterized, little is known about how dermal papilla's physical architecture is organized to support epithelial stem cells. Here, we leveraged intravital longitudinal imaging, 3D analysis, and genetic manipulations to understand how dermal papilla fibroblasts remodel their niche architecture and orchestrate it with stem cells during hair follicle growth. We find that dermal papilla fibroblasts develop broad membrane protrusions to form an upward polarized niche architecture enclosed by stem cells. This remodeling process strengthens niche's architectural integrity such that dermal papilla remained enclosed even after genetic fibroblast depletion. Furthermore, active TGFβ signaling within fibroblasts is required to maintain the niche architecture. Strikingly, when dermal papilla fibroblasts failed to receive TGFβ signals, they relocated inside out from within the epithelium precociously, reorganizing an enclosed architecture to a sheath of niche cells around the epithelium. These relocated fibroblasts retained niche identity and dictated a redistribution of stem cells to the outside layer of the hair follicle epithelium. Despite the redistribution, these stem cells still underwent proliferation and maintained differentiation in a concentric organization. Nevertheless, the trajectory of stem cell differentiation became defective and generated shorter hair shafts. Altogether, our work reveals that niche cells remodel shapes collectively to organize an architecture with polarity and integrity. This remodeling process requires architectural maintenance by active niche cell signaling. It further highlights that niche architecture dictates stem cell location and function. Spatial reorganization of stem cells and progeny partially preserves organ function. We propose that different organ shapes and functions may result from transformable niche architectures and their scalable mechanic-chemical crosstalk with stem cells.

Funding Source: NIH, China Scholarship Council-Yale World Scholars Program, New York Stem Cell Foundation, Howard Hughes Medical Institute scholar awards
Keywords: hair follicle stem cell niche, tissue architecture remodeling, skin fibroblast dynamics

TOPIC: HEMATOPOIETIC SYSTEM

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TWO DISTINCT ISOFORMS OF MLLT3 BALANCE HUMAN HSC FATE DECISIONS

Vavilina, Anastasia D - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*
Calvanese, Vincenzo - *Lab for Molecular Cell Biology, University College London, UK*
Fares, Iman - *MCDB, University of California, Los Angeles, CA, USA*
Capellera-Garcia, Sandra - *MCDB, University of California, Los Angeles, CA, USA*
Aguade Gorgorio, Julia - *MCDB, University of California, Los Angeles, CA, USA*
Nadel, Brian - *MCDB, University of California, Los Angeles, CA, USA*
Ma, Feiyang - *MCDB, University of California, Los Angeles, CA, USA*
Jami-Alahmadi, Yasaman - *MCDB, University of California, Los Angeles, CA, USA*
Wang, Yanling - *MCDB, University of California, Los Angeles, CA, USA*
Shin, Juliana - *Cedars-Sinai, Los Angeles, CA, USA*
Goodridge, Helen - *Cedars-Sinai, Los Angeles, CA, USA*
Wohlschlegel, James - *MCDB, University of California, Los Angeles, CA, USA*
Mikkola, Hanna - *MCDB, University of California, Los Angeles, CA, USA*

Abstract: MLLT3 is a critical human hematopoietic stem cell (HSC) regulator whose expression is highly enriched in HSCs, but declines in culture. Maintaining MLLT3 levels in cultured cord blood (CB) HSCs results in expansion of transplantable HSCs without transformation or differentiation bias. Analysis of RNA-seq data and epigenetic marks associated with MLLT3 gene in human HSCs revealed a second TSS linked to a novel MLLT3 isoform (MLLT3-S). MLLT3-S is predicted to encode a truncated protein that retains the C-terminal AHD domain responsible for protein-protein interactions, but lacks the N-terminal chromatin-binding YEATS domain. Presence of MLLT3-S transcript in human HSCs was verified using RNA-seq, q-RT-PCR and 5'RACE. Overexpression of MLLT3-S in cell lines confirmed that MLLT3-S interacts with known MLLT3 protein partners (DOT1L and superelongation complex), but is unable to bind chromatin. Although overexpression of MLLT3-L in cord blood (CB) HSCs promoted their expansion, overexpression of MLLT3-S did not, despite its highly HSC enriched expression, implying a different function in HSCs than that of MLLT3-L. Strikingly, while knockdown of MLLT3-L in CB HSCs abrogated the most undifferentiated HSPC and led to premature differentiation, knockdown of MLLT3-S resulted in immediate relative expansion of immunophenotypic HSCs. Accordingly, scRNA seq analysis of knockdown HSPCs showed opposite effects of the two isoforms on genes associated with HSC expansion (HLF, ERG, MSI2, PROCR etc.). scRNA and bulk RNA seq analysis of human developmental tissues revealed that MLLT3-L is expressed already in hemogenic endothelium (HE) in the embryo, whereas MLLT3-S first appears in AGM HSPCs after emergence from HE, and increases drastically in maturing fetal liver HSCs. RNA-seq analysis also revealed that overexpression of MLLT3-S suppresses IGFBP2, a regulator associated with HSC expansion and highly proliferative fetal HSCs, whereas MLLT3-L promotes its expression. These data suggest that by suppressing MLLT3-L-driven expression of IGFBP2 and

HSC expansion, MLLT3-S may promote HSC maturation in the fetal liver and their transition to homeostatic state. The interplay between two distinct isoforms of MLLT3 in human HSCs may provide a key mechanism by which mature HSCs balance between expansion and maintenance modes.

Funding Source: NIH RO1 DK100959 RO1 DK121557 and Ruth L. Kirschstein National Research Service Award T32HL069766 for A.V.
Keywords: hematopoietic stem cells, maintenance, maturation

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QUALITY ASSURANCE OF HEMATOPOIETIC STEM CELLS BY MACROPHAGES DETERMINES ADULT STEM CELL CLONALITY

Wattrus, Sam J. - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Smith, Mackenzie - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Pessoa Rodrigues, Cecilia - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Hagedorn, Elliott - *Section of Hematology and Medical Oncology, Boston University, Boston, MA, USA*
Zon, Leonard - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*

Abstract: Tissue stem cells persist for a lifetime and differentiate to maintain homeostasis. Despite their importance, there are no described quality assurance mechanisms for newly formed stem cells. We followed hematopoietic stem/progenitor cell (HSPC) development in zebrafish embryos and observed intimate and specific interactions between macrophages and HSPCs. We found that 70% of nascent HSPCs were contacted for 5 - 45 minutes by macrophages in the niche. Macrophage-HSPC interactions either lead to removal of cytoplasmic material and stem cell division or complete HSPC engulfment. 81% of all HSPC divisions occurred within 30 minutes of macrophage interaction. To assess if interactions affected hematopoietic stem cell (HSC) clone number, we depleted embryonic macrophages in a brainbow color barcoding system. Macrophage depletion with either the irf8 morpholino or clodronate liposomes significantly reduced the number of HSC clones (14 vs 24.6 clones ($p = 0.0002$)). To identify signals involved in interactions we pursued few-cell proteomics of macrophages marked by uptake of fluorescent HSPC material. This revealed enrichment of three calreticulin paralogs (calr, calr3a, and calr3b). Calreticulin is typically an ER-resident chaperone protein but can also be displayed on the cell surface as an "eat me" signal. Antibody staining confirmed surface Calreticulin on HSPCs, and single cell RNA-seq identified macrophage expression of lrp1ab and c1qa, the canonical surface Calreticulin binding partners. Morpholino knock down of calr3a or calr3b reduced macrophage interactions as much as 2-fold ($p = 0.0008$) and overexpression of non-ER bound calr3a or calr3b increased interactions up to 6-fold ($p = 0.0001$). Knock down of calr3a or calr3b in brainbow embryos also reduced HSC clone number (15.6 vs 19.6 clones ($p < 0.0001$)). Single cell RNA-seq of irf8 morphants identified a population of HSPCs marked by FOXO activity and a loss of ERK/MAPK target genes, suggesting that reactive oxygen species (ROS) high cells accumulated in the absence of macrophages. ROS levels correlated with Calreticulin staining by flow cytometry ($p < 0.0001$) and treatment with an ROS inhibitor reduced macrophage-HSPC interactions ($p < 0.05$). Our work supports a model in which embryonic macrophages determine HSC clonality by monitoring stem cell quality.

Funding Source: 1F31HL149154-01
Keywords: hematopoietic stem cell, macrophage, niche

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AN OLD CELL WITH NEW TRICKS: AN AGE-SPECIFIC PLATELET DIFFERENTIATION PATHWAY ARISING FROM HEMATOPOIETIC STEM CELLS

Poscablo, Donna M. - *Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA, USA*
Worthington, Atesh - *Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA, USA*
Smith-Berdan, Stephanie - *Institute for the Biology of Stem Cells, University of California, Santa Cruz, CA, USA*
Forsberg, Camilla - *Institute for the Biology of Stem Cells, University of California, Santa Cruz, CA, USA*

Abstract: Age-related changes to the hematopoietic system perturb platelet (Plt) hemostasis and leads to increased risk for cardiovascular and thrombotic diseases in the elderly. Therefore, our goal is to understand the mechanisms of age-related changes to the hematopoietic system. We have recently reported on the surprising fundamental differences between youthful and aging megakaryopoiesis described by a gain of expansion and reconstitution potential of MkPs during aging. These unexpected findings led us to hypothesize that aging leads to alterations to Plt-specific differentiation pathways. Using the FlkSwitch lineage tracing mouse model, we made the exciting discovery that Plt specification shifts from a canonical differentiation path in young mice to a Plt-specific unilineage pathway in old mice. All other hematopoietic lineages differentiate through the youth-like pathway. This unique Plt differentiation pathway resulted in two distinct populations of Plts in the Old mice and are derived via an age-specific pathway that includes MkPs but none of the canonical myeloid progenitor cell. Transplantation analysis of young and the two coexisting MkP populations revealed that the age-specific Plt pathway is propagated by the MkP compartment, with enhanced expansion potential, but not long-term self-renewal, of the unique age-specific old MkPs. Importantly, the functional differences of the age-specific Plt pathway are paralleled by unique molecular regulators of the age-specific old MkPs determined by RNA-sequencing analysis, providing target for controlling Plt production towards a more youthful pathway. Together, the age-specific Plt pathway demonstrate alternative mechanisms of Plt production throughout life and provides a putative explanation for increased risk for age-related Plt disorders and comorbidities.

Keywords: Age-Related Hematopoiesis, Megakaryocyte Progenitor, Hematopoietic Stem Cell

TOPIC: LIVER

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INDUCTION OF HEPATOCYTE DIFFERENTIATION FROM MOUSE LIVER DUCTAL ORGANOIDS BY TRANSDUCING A GROUP OF TRANSCRIPTION FACTORS

Tomofuji, Katsuhiro - *Department of Clinical Bio-resource Research and Development, Kyoto University, Kyoto, Japan*
Kondo, Jumpei - *Molecular Biochemistry and Clinical Investigation, Osaka University, Osaka, Japan*
Fukumitsu, Ken - *Surgery, Kyoto University, Kyoto, Japan*
Ishii, Takamichi - *Surgery, Kyoto University, Kyoto, Japan*
Hatano, Etsuro - *Surgery, Kyoto University, Kyoto, Japan*
Inoue, Masahiro - *Department of Clinical Bio-resource Research and Development, Kyoto University, Kyoto, Japan*

Abstract: Primary mature hepatocytes have limited proliferation ability and quickly lose physiological function in vitro. Therefore, alternative hepatocyte sources are expected for regenerative medicine and in vitro studies. Liver ductal organoids (LDOs), which was first reported in 2013, consists of liver tissue stem cells with bipotential capacity to differentiate to both hepatocyte and cholangiocyte lineages. However, LDOs strongly retain cholangiocyte characteristics in vitro, and current methods are insufficient to generate functionally mature hepatocyte from LDOs. In this study, we extracted twelve hepatocyte-enriched transcription factors as candidates to induce hepatocyte differentiation by comparing gene expression in murine LDOs and liver tissue. Using Tet-On inducible lentiviral vectors, we overexpressed each transcription factor and evaluated the expression of hepatocyte markers. Four out of these twelve factors, including Prox1 and Hnf4a, promoted hepatic lineage marker expression and downregulated cholangiocyte markers. Combination of these 4 transcription factors induced hepatocyte differentiation better than the conventional method; not only enhancing expression of hepatocyte markers but also hepatic functions such as lipid metabolism and xenobiotic/drug detoxification. These results suggest that induction of transcription factors is a promising strategy to generate functionally mature hepatocyte, and the induced LDOs by our method can be useful as an alternative hepatocyte source.

Keywords: Liver organoids, Hepatocyte differentiation, Transcription factors

TOPIC: MUSCULOSKELETAL

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MACROPOROUS HYDROGEL SCAFFOLD ENHANCES STEM CELL TREATMENT OF VOLUMETRIC MUSCLE LOSS

Eugenis, Ioannis - *Bioengineering, Stanford University, San Carlos, CA, USA*
Wu, Di - *Neurology, Stanford University, Palo Alto, CA, USA*
Hu, Caroline - *PAVIR, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA*
Chiang, Gladys - *PAVIR, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA*
Huang, Ngan - *Cardiothoracic Surgery, Stanford University, Palo Alto, CA, USA*
Rando, Thomas - *Neurology, Stanford University, Palo Alto, CA, USA*

Abstract: Volumetric muscle loss (VML), characterized by an irreversible loss of skeletal muscle due to trauma or surgery, is accompanied by severe functional impairment and long-term disability. Tissue engineering strategies using stem cells and biomaterials hold great promise for skeletal muscle regeneration. Hydrogels are attractive biomaterials for muscle regeneration given their simplicity and biocompatibility for cell delivery. However, conventional hydrogels generally lack macroporosity, which often delays host cell infiltration, transplanted cell proliferation, and new tissue formation. To overcome these limitations, we engineered a decellularized extracellular matrix hydrogel with a highly interconnected macropore matrix. Our results show that macroporosity significantly enhanced the survival and proliferation of transplanted muscle stem cells over 4 weeks in a mouse VML model. In addition, the macroporous scaffold facilitated rapid host cell infiltration, improved vascularization, and enhanced new muscle fiber regeneration. We are currently investigating the scalability of our approach in a rat VML model. Together, these results validate macroporous hydrogels as novel scaffolds for VML repair and skeletal muscle regeneration.

Keywords: Biomaterials, Muscle, Scaffold

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ELUCIDATING NEW THERAPEUTIC TARGETS FOR SKELETAL AGING BY DECODING HUMAN SKELETAL STEM CELL DIVERSITY DURING DEVELOPMENT AND REGENERATION

Ambrosi, Thomas H. - *Surgery - Stem Cell Institute, Stanford University, Stanford, CA, USA*
Taheri, Sahar - *Department of Computer Science and Engineering, University of California San Diego, CA, USA*
Sinha, Rahul - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Longaker, Michael - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Weissman, Irving - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Sahoo, Debashis - *Department of Computer Science and Engineering, University of California San Diego, CA, USA*
Chan, Charles - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*

Abstract: The rising socioeconomical burden of skeletal disorders due to age combined with the lack of effective therapeutics demands new approaches to prevent bone loss and regenerate skeletal tissues. Skeletal stem cells have emerged as a promising target for new treatment strategies to counter the age- and disease-related decline of bone health. However, progress has been hampered by the lack of knowledge about the identity and function of homogeneous skeletal stem cell (SSC) populations in humans. Here, we took advantage of our recently characterized human SSC to conduct comprehensive functional and SmartSeq2 single cell RNA-sequencing analysis of over 5,000 freshly, flow cytometrically purified human SSCs (hSSCs) in the context of development, during fracture regeneration, and under pathological conditions. Transcriptional signatures of single hSSCs from ten distinct skeletal sites during human fetal development revealed the genetic basis of structural and functional differences between bone compartments. We also identified new markers of growth plate- and periosteum-resident hSSCs and confirmed their local-

ization in situ by RNAscope. By combining single cell transcriptomic data with the in vitro and in vivo functional screening of hSSCs derived from over 400 patients aged 13 to 96 years, we discovered that age-related changes in bone growth and bone regeneration disorders stem from a pathological shift in the lineage commitment of the hSSC pool. Our results suggest that alterations in hSSC diversity underlies the development of skeletal fragility and nonunions during aging and are central to diseases such as fibrous dysplasia as well as neoplastic skeletal diseases. We developed an unbiased AI-based Boolean algorithm for single cell transcriptomic data to uncover gene regulatory networks of parallelly functionally tested patient SSCs, unraveling new therapeutic targets for stem cell-based regeneration. Specifically, we found that by combinatorial targeting of gene activity in shared gene regulatory networks between functional and dysfunctional hSSCs using small molecule agonists and inhibitors we could reinstate youthful bone-forming capacity in aged stem cells. In sum, this work offers new vantage points for designing urgently needed efficient strategies against skeletal aging and disease.

Keywords: Skeletal Stem Cell, Aging, Regeneration

TOPIC: EPITHELIAL_GUT

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THE TRANSPLANTATION OF COLON-DERIVED ORGANOID IMPROVES RADIATION-INDUCED COLITIS

Lee, Kyung Jin - *ORGANOIDSCIENCES, Korea*
Choi, Woo Hee - *Research Center, ORGANOIDSCIENCES LTD., Seongnam, Korea*
Jee, JooHyun - *Biomedical, CHA University, Seongnam, Korea*
Jeon, Hyemi - *Research Center, ORGANOIDSCIENCES LTD., Seongnam, Korea*
Lim, Taegyu - *Research Center, ORGANOIDSCIENCES LTD., Seongnam, Korea*
Seo, Jae-Hwi - *Research Center, ORGANOIDSCIENCES LTD., Seongnam, Korea*
Yoo, Jongman - *Research Center, ORGANOIDSCIENCES LTD., Seongnam, Korea*

Abstract: Radiation-induced colitis is a disease which occurs as the side effect of radiation therapy for pelvic malignancies. Collateral cells, which are damaged when irradiating cancer cell of other adjacent organs are known to be the main cause of radiation-induced colitis. Currently, conservative treatments are widely used to mitigate inflammatory symptoms, but direct recovery for damaged colonic epithelium is limited. EGFP-expressing colon-derived organoids were derived from colon tissue of CAG-EGFP mice (C57BL/6) for cell tracking. Radiation-Induced Colitis model was designed by irradiating 50 Gy on C57BL/6 mice's rectum, and the transplantation was performed from EGFP-expressing colon-derived organoids to mice colonic epithelium. GFP expression at colonic epithelium was confirmed by tissue autopsy at 4 weeks after post-transplantations, and histological analysis was performed to check successful EGFP-colon organoids' engraftment and differentiation. As a result, when EGFP-expressing colon-derived organoids were transplanted to radiated colitis mice, they were successfully engrafted to colonic epithelium and formed the mucosal layer reconstruction. For clinical application, we used fibrin glue application instead of Matrigel and cryopreservation to keep for long duration. The healing effect was confirmed by the engraftment of colon-derived organoids in the injured mucosal layer. In this study, colon-derived organoids obtained from adult stem cells were transplanted to irradiated rectum surface to promote mucosal layer regeneration.

Keywords: cell therapy, Proctitis, colonoid

TOPIC: NEURAL

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VISUALIZATION AND IN SILICO MODELING OF INTRANASALLY-ADMINISTERED HUMAN NEURAL STEM CELL MIGRATION FROM OLFACTORY BULB TO TRAUMATIC BRAIN INJURY SITES IN A RAT MODEL

Gutova, Margarita - *Stem Cell Biology and Regenerative Medicine, City of Hope National Cancer Center and Beckman Research Institute, Duarte, CA, USA*
Amirbekyan, Mari - *Stem Cell Biology and Regenerative Medicine, Beckman Research Institute, Duarte, CA, USA*
Cheng, Jeffrey - *Physical Medicine & Rehabilitation, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
Adhikarla, Vikram - *Computational and Quantitative Medicine, Beckman Research Institute, Duarte, CA, USA*
Barish, Michael - *Stem cell Biology and Regenerative Medicine, Beckman Research Institute, Duarte, CA, USA*
Hansen, Austin - *Matematics, University of California, Riverside, CA, USA*
Cho, Heyrim - *Mathematics, University of California, Riverside, CA, USA*
Rockne, Russell - *Computational and Quantitative Medicine, Beckman Research Institute, Duarte, CA, USA*
Bondi, Corina - *Physical Medicine & Rehabilitation, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
Kline, Anthony - *Physical Medicine & Rehabilitation, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*

Abstract: Effective stem cell-based therapies for traumatic brain injury (TBI) depends on efficient delivery to the affected areas. Immortalized L-myc expressing human neural stem cells (hNSCs; LM-NSC008) display an inherent tropism to sites of damage after intranasal (IN) administration, and thus can potentially be exploited for cell replacement and regeneration strategies. The effectiveness of hNSC-mediated therapies requires successful engraftment, which in turn depends in part on the number of hNSCs reaching the target site. We hypothesize that IN hNSCs will migrate toward injury sites in an organized manner and contribute to cell replacement and regeneration, as well as neuroprotection via secretion of neurotrophins and induction of host neurogenesis. Defining the processes underlying migration of hNSCs to injury sites thus becomes critical to achieving optimally effective therapies. Here we demonstrate visualization of the distribution of LM-NSC008 cells in optically cleared tissue after multiple IN injections beginning 7 days post-TBI in an immunocompetent rat model of controlled cortical impact [CCI] injury. eGFP-expressing LM-NSC008 cells were administered in six IN injections of 1x106 cells in 25ml saline every other day. Daily cyclosporin A (10 mg/kg; s.c.) injections started on day 5 post-TBI prevented immune rejection of the hNSCs. 5-8 weeks after TBI, and 4-7 weeks after initiating administration of LM-NSC008 cells, rat brains were harvested, and 1mm-thick coronal sections of fixed brain were cleared with CLARITY, immunostained, and imaged at TBI and contra sites. NSCs were quantified across 7 sections from male and female rat brains. We observed NSC migration along white

matter tracts towards TBI sites. These migration routes were predictable depending on the spatial relationships of hNSC administration sites, the locations of TBI targets, and the organization of intervening white matter tracts. Further, in silico simulations performed using a human DT-MRI atlas using the olfactory bulbs as the source of hNSCs agreed with our observations. Our work serves as proof of concept for the use of cell-mediated therapies for the treatment of TBI and provide a strong rationale for using DT-MRI together with computational simulations for predicting and optimizing NSC-mediated therapies.

Funding Source: 1R01NS121037-01
Keywords: We aim to optimize intranasal delivery of allogeneic LM-NSC008 cells to treat traumatic brain injury and to achieve therapeutic efficacy both alone and when combined with neurorehabilitation to improve the treatment of TBI patients., Visualization of LM-NSC008 cells using CLARITY and development of computational model of NSC migration towards Traumatic Brain Injury in the brain., NSC, TBI, CLARITY, Intranasal administration

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PROMOTING RECOVERY IN A MOUSE MODEL OF ISCHEMIC STROKE USING HUMAN CELL TRANSPLANTATION AND CELL SURVIVAL BASED INTERVENTIONS TO ENHANCE NEUROPLASTICITY

Islam, Rehnuma - *Institute of Medical Science, University of Toronto, Scarborough, ON, Canada*
Drecun, Stasja - *Institute of Biomedical Engineering, University of Toronto, ON, Canada*
Varga, Balazs - *Wellcome Trust Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, UK*
Vonderwalde, Ilan - *Institute of Biomedical Engineering, University of Toronto, ON, Canada*
Siu, Ricky - *Department of Surgery, University of Toronto, ON, Canada*
Nagy, Andras - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*
Ahlfors, Jan-Eric - *New World Laboratories, Laval, QC, Canada*
Morshead, Cindi - *Institute of Medical Science, University of Toronto, ON, Canada*

Abstract: Current therapeutics to promote stroke recovery are limited, leading to debilitating, long-term outcomes for stroke patients. Rehabilitation is a primary course of action to enhance neuroplasticity, albeit with limited efficacy. Harnessing the potential of neural precursor cells (NPCs), through cell transplantation or activation of endogenous NPC populations, are promising avenues to improve stroke outcomes and both strategies have achieved some success. Herein we have explored optimization of these approaches by (1) determining an optimal cell source for transplantation and (2) enhancing endogenous cell survival post-stroke, including resident brain NPCs. We studied our approaches in a model of sensory-motor cortical stroke using the potent vasoconstrictor, endothelin-1 (ET-1) to induce a reproducible cortical lesion that results in motor deficits. We examined a human derived NPC population that is generated in vitro from pluripotent stem cells and has the capacity to generate cortically specified neural cells. Mice transplanted with human NPCs in the subacute phase post-stroke demonstrated motor recovery, however, hNPC maturation and integration was not necessary for the observed improvements. This suggested that recovery is correlated with host neuroplasticity and we predicted that improved NPC activation and cell survival of endogenous cells may underlie the improvement. To improve endogenous cell survival, a proprietary drug, NWL283, was used to inhibit caspase-3/7 induced cell death. NWL283 administration starting at the time of ET-1 stroke and for 8 days, resulted in increased neuronal survival, increased NPCs and reduced microglia activation and improved functional outcomes. Together, these findings suggest that we can target both exogenous and endogenous neural precursor cells to promote neuroplasticity in the stroke injured cortex.

Keywords: Stroke, Neural stem cells, Neuroplasticity

TOPIC: PANCREAS

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ROBUST ESTABLISHMENT AND EXPANSION OF HUMAN PANCREATIC DUCT ORGANOIDS IN PANCREACULT ORGANOID MEDIUM KITS

Quiskamp, Nina - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Tsai, Angela - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Pan, Joanna - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Hasan, Misha - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Chang, George - *Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada*
Tai, Isabella T. - *Canada's Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, BC, Canada*
Eaves, Allen C. - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon A. - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Conder, Ryan K. - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: Organoids are a novel in vitro culture system that promotes the growth of primary and PSC-derived cells in three-dimensional culture to generate structures that recapitulate tissue morphology. We have developed PancreaCultTM Organoid Medium Kits (Human), which combine PancreaCultâ„¢ Organoid Initiation Medium (OIM) and serum-free PancreaCultâ„¢ Organoid Growth Medium (OGM) into a robust and standardized workflow for the establishment and expansion of pancreatic duct organoids. To establish organoids, fresh or cryopreserved islet-depleted exocrine tissue was seeded in CorningÂ® MatrigelÂ® domes and cultured in PancreaCultâ„¢ OIM. After 3 days, the medium was changed to PancreaCultâ„¢ OGM, and the organoids were passaged and maintained in this medium thereafter. Organoids were passaged as fragments every 4 - 7 days. Organoids were successfully established from 5 out of 5 donors with a 2- to 6-fold expansion in cell numbers every 7 - 14 days. Real-time PCR andimmunofluorescence microscopy demonstrated that organoids expressed pancreatic duct markers (PDX1, SOX9, KRT19, CFTR, CA2, and MUC1), as well as the proliferation marker Ki-67 and the stem cell gene LGR5. Pancreatic duct organoids could be maintained for at least 10 passages (n = 3) and cryopreserved as fragments that could be used for rapid re-establishment of cultures at later time-points. In addition to normal pancreatic duct cultures, PancreaCultâ„¢ OGM supported the efficient long-term expansion of three pre-established pancreatic ductal adenocarcinoma (PDAC) organoid lines and could be modified to suppress normal

cell growth to select for tumor cells with activating KRAS mutations. Our results demonstrate that PancreaCultTM Organoid Medium Kits provide a robust and flexible in vitro culture system for the establishment and expansion of normal pancreatic duct cells as well as the long-term expansion of PDAC organoids.

Funding Source: MITACS Accelerate Internship
Keywords: Organoids, Pancreatic Ductal Adenocarcinoma (PDAC), Pancreatic Duct

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

633

IDENTIFICATION OF NOVEL MLLT3 PROTEIN INTERACTIONS THAT GOVERN HUMAN HSC SELF-RENEWAL

Fares, Iman - *Molecular, Cell and Developmental Biology, University of California, Los Angeles, Glendale, CA, USA*
Jami, Yasaman - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Calvanese, Vincenzo - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Ma, Feiyang - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Wang, Yanling - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Shin, Juliana - *Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Goodridge, Helen - *Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Wohlschlegel, James - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*
Mikkol, Hanna - *Molecular Cell and Developmental Biology, UCLA, Los Angeles, CA, USA*

Abstract: MLLT3 is a critical regulator of human hematopoietic stem cell (HSC) self-renewal. We showed that MLLT3 expression is enriched in human HSCs but reduced upon culture, leading to loss of HSC activity. Restoring physiological MLLT3 levels in cultured cord blood (CB) hematopoietic stem and progenitor cells (HSPCs) mitigates this issue and allows expansion of transplantable human HSCs without blocking differentiation or causing transformation. Mechanistic studies showed the MLLT3 co-operates with DOT1L to sustain H3K79me2, a gene body- associated active mark that protects the HSC transcriptional program and thereby enables symmetric self-renewal in culture. However, the DOT1L dependent mechanism does not explain how all MLLT3 target genes are regulated, as some gene groups such as histone genes are downregulated by MLLT3 and show minimal H3K79me2 in MLLT3 binding sites. We hypothesize that MLLT3 binds distinct groups of target genes in HSPCs as part of different protein complexes. To test this hypothesis, we performed MLLT3 immunoprecipitation followed by mass spectrometry (IP-MS) in cultured human CB HSPC. We first optimized MLLT3 IP-MS using KG1 AML cell line which shows comparable MLLT3 protein level, and similar expression of many MLLT3 target genes and surface phenotype as HSPC. In addition to detecting the known MLLT3 interactors such as DOT1 (DOT1L, MLLT10) and super elongation complex (AFF1, AFF4) in human HSPCs, the IP-MS data revealed novel MLLT3 interacting complexes such as the NURD, Cohesin, NCOR and SWI/SNIF complexes, which we validated using co-IP and western blot. We further showed that, although many MLLT3 complex partners can co-bind with MLLT3 to its target genes, these are separate complexes with MLLT3 as the common interactor. These data suggest that MLLT3 governs HSC fate decisions by interacting with diverse protein complexes that may help coordinate the dynamics by which different HSC regulatory genes are transcribed, and link transcription elongation to other critical regulatory functions in HSCs such as chromatin remodeling, chromosomal looping and genome integrity. In addition to providing new perspectives to human HSC self-renewal regulation, this work represents a technical tour-de-force, as such proteomic studies of HSC regulators have not been conducted on primary human HSCs.

Funding Source: This work was supported by BSCRC post-doctoral fellowships and T32 HL-086345-13 506 Developmental Hematology fellowship for IF and R01DK100959, R01DK121557, Rally Foundation, and BSCRC and JCCF seed grant for HKAM.
Keywords: Hematopoietic stem cell expansion, HSC self-renewal, MLLT3

TOPIC: PLURIPOTENT STEM CELLS

635

NICOTINE ACUTE EXPOSURE HINDERS HUMAN DENTAL PULP STEM CELLS PROLIFERATION, MIGRATION AND SURVIVAL POTENTIALS

Tahrani Hardin, Leyla - *Biomedical Sciences, University of the Pacific, San Francisco, CA, USA*
Vang, David - *Biomedical Sciences, University of the Pacific, San Francisco, CA, USA*
Xiao, Nan - *Biomedical Sciences, University of the Pacific, San Francisco, CA, USA*

Abstract: According to CDC, in 2019 nearly 40 million U.S. adults still smoke cigarettes, and this costs the United States many billions to treat smoking-related diseases. Many previous studies demonstrated that nicotine could impede the regenerative potential of several types of cells including stem cells. Dental pulp stem cells (DPSCs) are known for their multipotent differentiation, easy extraction, low immunogenicity, and a high self-renewal ability. Therefore, they constitute promising stem cells sources for various clinical applications. We studied whether the nicotine could impact DPSCs regenerative properties. Migration ability was tested by scratch test. We exposed DPSCs to different concentrations of nicotine (100 nM, 1 µM, 10 µM, 100 µM, 1 mM) in serum-free medium. After scratch, we measured the wound area for 48 hours. The effect of nicotine (100 nM, 1 µM, 10 µM, 100 µM, 1 mM, and 10 mM) on cell proliferation was measured using WST1 test for 4 days. The survival potential was determined by clonogenic test using two different cell numbers (2 x 103 and 2 x 104 cells) with nicotine treatment before cell seeding for 10 days. The results indicated that low concentrations of nicotine (100 nM, 1 µM, and 10 µM) showed to not impede DPSCs proliferation or migration, but high concentrations (1 mM) significantly ($p< 0.05$) inhibited DPSCs migration. We significantly found a reduced cell survival rate after nicotine treatment; the colony formation ability decreased in dose-dependent manner. 10 mM of nicotine showed to totally ($p< 0.05$) inhibit the colony formation at low or high seeded cell numbers (2 x 10^3 and 2 x 10^4 cells). WST1 test showed that 10 mM is a cytotoxic concentration for DPSCs. Results of this study provide further evidence that nicotine at high

concentrations, a major compound of cigarette, may affect the regenerative potentials of human DPSCs.

Funding Source: This work was supported by Tobacco-Related Diseases Research Program Grant T30IP0917 from Regents of the University of California.
Keywords: Proliferation survival migration, Cigarette smoking, nicotine, acute exposure, Dental pulp stem cells

TOPIC: CARDIAC

863

LOSS OF SNORD116 LNCRNA REDUCED PATHOLOGICAL REMODELING AFTER MYOCARDIAL INFARCTION IN MICE

Pilcher, Lucy - *Medicine, University of Vermont, Colchester, VT, USA*
McElroy-Yaggy, Keara - *Medicine, University of Vermont, Colchester, VT, USA*
Soloman, Laura - *Medicine, University of Vermont, Colchester, VT, USA*
Spees, Jeffrey - *Medicine, University of Vermont, Colchester, VT, USA*

Abstract: Myocardial infarction is the leading cause of morbidity and mortality worldwide. Pathological remodeling takes place in the weeks following myocardial infarction (MI), wherein the heart undergoes hypertrophy and fibrosis, leading to stiffened ventricular walls. Aerobic exercise causes physiological or beneficial remodeling, during which the heart becomes more efficient. We hypothesized that genes highly expressed in an exercised heart would be beneficial to cardiac remodeling after MI. We performed a cDNA microarray to identify differentially-expressed genes in epicardial cells from running mice when compared with cells from healthy, non-running controls. Snord116 was the most differentially-expressed gene (increased) after 1 week of running exercise. The Snord116 locus produces a long-noncoding RNA called Snord116hg, which regulates the expression of more than 2,400 metabolism-related genes through DNA methylation. We obtained the Snord116 knock out mouse (snord116p-), performed myocardial infarction surgery, and used echocardiography to monitor cardiac remodeling and function after MI. Starting at 4 weeks post-MI, the left ventricular diameter of Snord116p- mice was significantly smaller than that of aged-matched, wild type littermate (WT LM) controls. At 8 weeks post-MI, the Snord116p- mice had increased anterior cardiac wall thickness and significantly improved cardiac function, measured by ejection fraction and wall motion score. The Snord116p- mice also had significantly less scar tissue formation, measured by percent area collagen (trichrome stain). The Snord116p- mice experienced less hypertrophy and fibrosis, demonstrating that loss of Snord116 reduces pathological remodeling and improves cardiac function after MI. Snord116 has not previously been studied in the heart, but may be an important target to regulate cardiac remodeling after injury.

Funding Source: NIH R01 HL132264, UVM CVRI ECAC grant
Keywords: Cardiac remodeling, lncRNA, Cardiac function

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

865

STUDY OF SURFACE CURVATURE EFFECT ON IPSC-DERIVED ENDOTHELIAL CELL DIFFERENTIATION AND CULTURE ON DIFFERENT MATERIALS

Park, Dongjeong - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer IBMT, Wuerzburg, Germany*
Gepp, Michael - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer IBMT, Wuerzburg, Germany*
Hasselmann, Sebastian - *CeSMA – Center Smart Materials & Adaptive Systems, Fraunhofer ISC, Wuerzburg, Germany*
Steenhusen, Sönke - *CeSMA – Center Smart Materials & Adaptive Systems, Fraunhofer ISC, Wuerzburg, Germany*
Neubauer, Julia - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer IBMT, Wuerzburg, Germany*
Zimmermann, Heiko - *Head of Institute, Fraunhofer IBMT, Wuerzburg, Germany*

Abstract: Since the last decade, induced pluripotent stem cells (iPSCs) have been regarded as a potential source of patent-specific endothelial cells (ECs). However, two-dimensional endothelial differentiation and the followed conventional cultivation in a static environment have limitations that cannot mimic the natural endothelial cell niche. Therefore, we hypothesized that more physiological approaches, such as modification for surface curvature and reduced stiffness, are able to overcome these limitations. In this work, we used spherical alginate hydrogel microcarriers, which offer curvature (approx. k=220 µm-1 to 380 µm-1) and modified with Matrigel™ with or without tyramine-linker. The differentiation process on microcarriers was carried out in suspension culture and compared to 2D differentiation. Our recent experimental data proved that the differentiated ECs on modified alginate showed enhanced proliferation and strong increase in endothelial gene expression as well as functional characteristics. Moreover, iPSC-ECs further suppressed Endothelial-to-Mesenchymal Transition (EndMT)-related genes and showed changes in gene expression involved in mitochondrial integrity and metabolism in an adapted medium. For further potential applications (e.g. vascularized organoids), we studied the behavior of iPSC-ECs on 3D structured surfaces made of inorganic-organic hybrid polymers (ORMOCER®s). The versatile material class of ORMOCER®s is based on a silica backbone that is extended by organic components depending on the desired material properties. We exploited to generate structures with a various curvature via two-photon polymerization to mimic vascular structure. Biocompatibility features and the expression of endothelial genes of iPSC-ECs on ORMOCER® showed the high potential of ORMOCER® as an endothelial scaffold. These results lead to the conclusion that both, mechanical properties and curvature positively influence endothelial development and sustainable culture.

Funding Source: Funding Source: This work was supported by the Bavarian Ministry of Economic Affairs, Regional Development and Energy.
Keywords: 3D endothelial differentiation, Alginate hydrogels, ORMOCER®

TOPIC: EPITHELIAL_SKIN

867

CONVERSION INTO MELANOCYTES WITH OPTIMAL FACTORS AND SYSTEM

Zhang, Yixuan - *Medical School, Jiangsu University, Zhenjiang, China*
Li, Yumei - *Medical School, Jiangsu University, Zhenjiang, China*
Zheng, Yunwen - *Faculty of Medicine, University of Tsukuba, Japan*

Abstract: The loss of function of melanocytes leads to vitiligo, which seriously affects the physical and mental health of the affected individuals. Presently, there is no effective long-term treatment for vitiligo. Therefore, it is imperative to develop a convenient and effective treatment for vitiligo. Regenerative medicine technology for direct reprogramming of skin cells into melanocytes seems to be a promising novel treatment of vitiligo. This involves the direct reprogramming of the patient's skin cells into functional melanocytes to help ameliorate the loss of melanocytes in patients with vitiligo. However, this method needs to be first tested on mice. Although direct reprogramming is widely used, there is no clear protocol for direct reprogramming into melanocytes. Moreover, the number of available transcription factors is overwhelming. Here, a concentrated lentivirus packaging system protocol is presented to produce transcription factors selected for reprogramming skin cells to melanocytes, including Sox10, Mitf, Pax3, Sox2, Sox9, and Snai2. Mouse embryonic fibroblasts (MEFs) were infected with the concentrated lentivirus for all these transcription factors for the direct reprogramming of the MEFs into induced melanocytes (iMels) in vitro. Furthermore, three transcription factors of Sox10, Mitf, and Pax3 were screened for their important roles, and the system was optimized for direct reprogramming to melanocytes. The expression of the characteristic markers of melanin in iMels at the gene or protein level was significantly increased. These results suggest that direct reprogramming of fibroblasts to melanocytes could be a successful new therapeutic strategy for vitiligo and confirm the mechanism of melanocyte development, which will provide the basis for further direct reprogramming of fibroblasts into melanocytes in vivo.

Funding Source: the National Natural Science Foundation of China (82070638 and 81770621) and the Natural Science Foundation of Jiangsu Province (BK20180281).
Keywords: Direct reprogramming, melanocyte, transcription factors

TOPIC: HEMATOPOIETIC SYSTEM

869

THE EPIGENETIC REGULATOR LANDSCAPE OF STEMNESS NETWORKS IN AML

Barbosa, Karina - *Tumor Initiation and Maintenance, Sanford Burnham Medical Research Institute, La Jolla, CA, USA*
Deshpande, Anagha - *Tumor Initiation and Maintenance Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Xiang, Ping - *Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada*
Minkina, Anna - *Department of Genome Sciences, Department of Genome Sciences, Seattle, WA, USA*
Schischlik, Fiorella - *Cancer Data Science Laboratory, National Cancer Institute, Bethesda, MD, USA*
Brown, Adam - *Genetic Perturbation Platform, Broad Institute, Cambridge, MA, USA*
Robertson, Neil - *Institute of Cancer Sciences, University of Glasgow, UK*
Doench, John - *Genetic Perturbation Platform, Broad Institute, Cambridge, MA, USA*
Adams, Peter - *Tumor Initiation and Maintenance Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Humphries, R. Keith - *Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada*
Ruppin, Eytan - *Cancer Data Science Laboratory, National Cancer Institute, Bethesda, MD, USA*
Shendure, Jay - *Department of Genome Sciences, University of Washington, Seattle, WA, USA*
Mali, Prashant - *Department of Bioengineering, University of California San Diego, CA, USA*
Deshpande, Aniruddha - *Tumor Initiation and Maintenance Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*

Abstract: Several leukemia-associated oncoproteins activate transcriptional circuits resembling a stem-like state in Acute Myeloid Leukemia (AML). This is achieved by enlisting the activity of specialized epigenetic machinery components. Using AF10-rearranged (AF10-R) AML as a paradigmatic example, we sought to comprehensively map epigenetic regulators critical for perpetuating these networks in AML, since they would provide attractive therapeutic targets. To this end, we used an endogenous MEIS1-GFP-reporter tagged U937 AML cell line and conducted a pooled, domain-focused CRISPR screen targeting >600 epigenetic regulators. Through this screen, we identified and independently validated multiple epigenetic regulators required for sustaining MEIS1 expression, including novel candidates such as TAF6, LDB1, KAT2A, AFF2, JADE3, CCDC101, and casein kinase 2 (CK2), in addition to previously characterized regulators such as DOT1L, AF10, ENL, and HBO1. A small, secondary pooled CRISPR screen, coupled with a single-cell RNA-seq readout (CROP-seq) revealed that CRISPR-deletion of several candidates not only reversed the expression of MEIS1 but also that of several "stemness" genes while activating the expression of differentiation-associated genes. Of particular interest to us was CK2, since inhibitors of this kinase are currently being tested in Phase II clinical trials. Our studies showed that CRISPR-knockout of CK2 components or pharmacological CK2 inhibition significantly reduced the proliferation of cells with distinct HOX-activating mutations. CRISPR-mediated CK2 deletion led to a significant reduction not only in the HOX/MEIS genes active in AF10-R AML, but also reversed activation of AF10-fusion targets as assessed by Gene Set Enrichment Analysis. Phospho-proteomic experiments demonstrated that CK2-mediated MEIS1 serine 196 phosphorylation was partially responsible for its leukemia-promoting effects. Taken together, CK2 inhibition led to a significant diminution of the "stemness" network activated by AF10-fusions, leading to potent anti-leukemia effects in mouse and human models of AML. Our studies show that CK2 controls the leukemia epigenome, in part through phosphorylation of MEIS1 and other epigenetic regulators necessary for activating stem-cell-associated genes in AML.

Funding Source: National Cancer Institute P30 CA030199, Rally Foundation, Luke Tatsu Johnson Foundation 19YIN45, Emerging Scientist of Children's Cancer Research Fund, V Foundation (TVF) DVP2019-015, Department of Defense W81XWH-20-1-0703.

Keywords: Acute Myeloid Leukemia, Epigenetics, CRISPR Screening

TOPIC: MUSCULOSKELETAL

871

MESENCHYMAL STROMAL CELLS AND THEIR SECRETOME IMPROVE REPAIR FOLLOWING OSTEOCHONDRAL INJURY IN A MURINE MODEL

Seah, Matthew - *Department of Surgery, University of Cambridge, UK*
Birch, Mark - *Department of Surgery, University of Cambridge, UK*

Abstract: Osteochondral injuries are a recognised factor in the development of osteoarthritis (OA). Mesenchymal stromal cells (MSCs) represent a promising biological therapeutic option as an OA-modifying treatment, and they also secrete factors that may have an anti-catabolic effect and/or encourage endogenous repair. We aim to study the effects of (i) intra-articular injection of human bone-marrow-derived MSCs and (ii) their secretome on recovery in a murine knee osteochondral injury model. The MSC secretome was generated by stimulating human bone-marrow-derived MSCs with tumour necrosis factor alpha (TNFα). Mice (n=48) were injected with i) MSC secretome, ii) MSCs or iii) cell culture medium (control). Pain was assessed by activity monitoring, and cartilage repair, subchondral bone volume and synovial inflammation were evaluated using histology and microCT. Both MSC- and MSC-secretome-injected mice showed significant pain reduction at day 7 when compared to control mice, but only the MSC-injected mice maintained a significant improvement over the controls at day 28. Cartilage repair was significantly improved in MSC-injected mice. No significant effects were observed with regards to synovial inflammation or subchondral bone volume. The MSC secretome demonstrates regenerative effects but this does not appear to be as sustained as a MSC cell therapy. Further studies are required to investigate if this can be overcome using different dosing regiments for injection of the MSC secretome. As we further understand the regenerative properties of the MSC secretome, we may be able to enhance the clinical translatability of these therapies. Direct intra-articular injection of MSCs for the treatment of OA also appears promising as a potential future strategy for OA management.

Funding Source: MS is funded by a grant from the Wellcome Trust (PhD Programme for Clinicians).
Keywords: mesenchymal, regeneration, cartilage

TOPIC: NEURAL

873

MELANOMA STEM CELLS ENCOURAGE THE POLARIZATION OF NEUTROPHILS BY PROMOTING THE N2 PHENOTYPE

Anselmi, Martina - *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*
Fontana, Fabrizio - *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*
Limonta, Patrizia - *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*
Marzagalli, Monica - *Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy*

Abstract: Emerging evidence suggests that cancer stem cells (CSCs) are involved in melanoma progression by reshaping the tumor microenvironment. In melanoma patients, poor prognosis often correlates with high presence of neutrophils, suggesting that tumors can recruit these immune cells and promote their switch towards a pro-tumorigenic phenotype (N2). However, how melanoma SCs dialog with neutrophils in their microenvironment has not been completely elucidated yet. The aim of the present study was to unravel the role of melanoma SCs in neutrophil polarization. Herein, we demonstrated that A375 CSC conditioned media (CSC-CM) can promote the switch of differentiated neutrophil-like HL60 (dHL60) cells from N1 to N2 phenotype, by inducing the activation of the ERK/STAT3 cascade via TGF-β and IL-6 secretion. Moreover, after exposure to CSC-CM, dHL60 cells were shown to release NETs, associated with increased production of ROS and H2O2 in a cell death-independent manner. Finally, CSC-CM-treated neutrophils exhibited an increased expression of CXCR-2 and CD66b as well as the activation of the NF-kB pathway, followed by the production of several pro-tumor soluble factors, including TNF-α, IL6 and MMP9. Collectively, our results suggest that melanoma SCs can reshape the immune system by triggering the N2 phenotype in neutrophils

Keywords: melanoma cancer stem cell, neutrophils, NET, ROS,Cytokine

TOPIC: PANCREAS

875

EFFECTIVE GENERATION OF FUNCTIONAL PANCREATIC B -CELLS FROM HUMAN DERIVED DENTAL STEM CELLS OF APICAL PAPILLA

Abuarqoub, Duaa - *Faculty of Pharmacy, University of Petra, Amman, Jordan*
Adwan, Sofia - *Faculty of Health Sciences, American University of Madaba, Amman, Jordan*
Aslam, Nazneen - *Cell Therapy Center, The University of Jordan, Amman, Jordan*
Zaza, Rand - *Cell Therapy Center, The University of Jordan, Amman, Jordan*
Jafar, Hanan - *Cell Therapy Center, The University of Jordan, Amman, Jordan*
Awidi, Abdalla - *Cell Therapy Center, The University of Jordan, Amman, Jordan*

Abstract: Diabetes Milletus Type 1 is an autoimmune disease that occurs due to the destruction of insulin producing cells (β cells), resulting in hyperglycemia. Therefore, diabetic patients depend on the insulin treatment for rest of their lives. One of the possible treatments for such kind of chronic and uncured disease could be the cell replacement therapy. Stem cells are considered a promising source to replace the nonfunctional beta cells in diabetic patients with functional and mature beta cells which are capable to perform efficiently. Hence, in this study we aimed to examine the potential of dental stem cells of apical papilla (SCAP) to differentiate into functional islet like cell aggregates (ICAs) by using three stages beta cell differentiation protocol. Our strategy was to induce the differentiation of SCAP into definitive endoderm. The success of endodermal differentiation was determined by measuring the expression of definitive endodermal markers; FOXA2 and SOX-17 by flow cytometry. Next, the maturity and functionality of the differentiated cells was evaluated by measuring the amount of insulin and C-peptide secreted by the derived ICAs

by using ELISA. Additionally, the expression of mature beta cell markers; Insulin, C-peptide, Glucagon, PDX-1 and Somatostatin, was detected by using confocal microscopy while the staining of the mature islets like clusters by using diphenythiocarbazone (DTZ). Our results have shown that SCAP were sequentially committed to definitive pancreatic endoderm, and β-cell like cells by upregulating the expression of FOXA2 and SOX17 significantly, compared to the control undifferentiated cells($p < 0.05$). Moreover, the identity of ICAs was confirmed by DTZ-positive staining, as well as by the expression of C-peptide, Pdx-1, insulin, and glucagon at day14. It was noted that at day14, differentiated-ICAs released insulin and C-peptide in a significant manner compared to the control undifferentiated group ($p < 0.05$, $p < 0.0005$) respectively, exhibiting in vitro functionality. Our results demonstrated for the first time that SCAP could be differentiated into pancreatic cell lineage, suggesting a new unambiguous and nonconventional source of stem cells derived from human tissue that could be used for stem cell therapy to treat type 1 diabetes.

Keywords: Diabetes, β-differentiation, Dental stem cells

TOPIC: PLURIPOTENT STEM CELLS

877

ANALYSIS OF THE ROLE OF OCIAD PROTEINS IN HUMAN HEMATOPOIETIC DEVELOPMENT

Kamat, Kajal - *Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, India*
Prakash, Aishwarya - *Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, India*
Inamdar, Maneesha - *Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru, India*

Abstract: Significant advances in in vitro differentiation and cellular reprogramming technologies have enabled de novo generation of multipotent hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs). However, efficient generation of adult-like HSCs with engraftable and multi-lineage reconstitution potential demands identification of additional regulators of hematopoiesis. Also, while HSC development is thought to involve several transient intermediates, reagents for identification and temporal mapping of these are limited. Mitochondrial activity is an important determinant of stem cell fate and function. We showed earlier that hPSC populations demonstrate a spectrum of metabolic sub-states, which can be strategically canalized to generate desired cell types. Further, the mitochondrial activity regulator Asrij/OCIAD1 affects these sub-states to control early mesoderm differentiation. However the role of its family member and interactor, OCIAD2, is not known. To understand how mitochondrial activity can dictate hematopoietic specification, we investigated the role of OCIAD proteins in human hematopoietic development. Hematoendothelial induction and hematopoietic progenitor numbers were sensitive to Asrij/OCIAD1 depletion by CRISPR-Cas9-mediated knockout as well as its constitutive overexpression. We similarly examined the role of OCIAD2, the family member and interactor of OCIAD1, which suggested a role in differentiation. We are also generating fluorescent reporter-expressing hPSC lines that will allow spatiotemporal analysis and capture intermediate stages prevalent during hematopoietic differentiation that may require the function of OCIAD proteins.

Keywords: Human pluripotent stem cells, Mitochondrial activity, Hematopoietic differentiation

POSTER SESSION II: EVEN

4:00 PM – 5:00 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

102

ΔNP63α TRANSCRIPTIONALLY REPRESSES P53 TARGET GENES IN RADIATION-INDUCED DNA DAMAGE RESPONSE

Kudo, Ken-ichi - *Radiation Life Sciences, Fukushima Medical University, Fukushima-City, Japan*
Tsuyama, Naohiro - *Radiation Life Sceiences, Fukushima Medical University, Fukushima, Japan*
Nagata, Kento - *Radiation Effects Research, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan*
Imaoka, Tatsuhiko - *Radiation Effects Research, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan*
Fukami, Miwa - *Radiation Life Sceiences, Fukushima Medical University, Fukushima, Japan*
Sugai-Takahashi, Misaki - *Radiation Life Sceiences, Fukushima Medical University, Fukushima, Japan*
Azami, Yusuke - *Medical Oncology, Fukushima Medical University, Fukushima, Japan*
Muramatsu, Moe - *Diagnostic Pathology, Fukushima Medical University, Fukushima, Japan*
Sakai, Akira - *Radiation Life Sceiences, Fukushima Medical University, Fukushima, Japan*

Abstract: Ionizing radiation is known to result in carcinogenesis through the induction of oxidative DNA damage, such as DNA double strand breaks. However, the DNA damage response (DDR) is a protective mechanism against oxidative DNA damage and may result in cell cycle arrest and apoptosis. p63 is a member of the p53 family and shares high homology with the DNA-binding domain of p53. ΔNp63α, one of the p63 splicing variants, is said to competitively inhibit the tumor suppressive effect of p53 and is constitutively expressed in the basal layer containing stem cells in stratified epithelial tissues, including the mammary gland and epidermis, where it plays a critical role in the maintenance of stemness and tissue development. However, the p53 repressive activity of ΔNp63α may cause genomic instability in stem cells. In this study, we confirmed the DDR inhibitory effect of ΔNp63α by siRNA knockdown experiments using human mammary epithelial cells and gene transfer experiments using human induced pluripotent stem (iPS) cells. It was confirmed that the mRNA/protein expression levels of BAX and p21 were significantly increased in p63-siRNA knockdown cells (sip63) after X-ray irradiation (4 Gy, 0.7 Gy/min), but not in scramble-siRNA (scr). In addition, flow cytometric analysis revealed an increase in apoptotic cells and a decrease in 5-ethynyl-2´-deoxyuridine uptake in sip63 compared to scr. Furthermore, RNA transcriptomic analysis based on the Gene Ontology biological process database showed that cell cycle-re-

lated expression was decreased and programmed cell death-related expression was increased in sip63 compared to scr. On the other hand, the ectopic expression of ΔNp63α in apoptosis-sensitive iPS cells reduced the expression levels of BAX post-irradiation and significantly decreased apoptotic cells induced by radiation. Taken together, these results indicate that ΔNp63α represses the p53-related radiation-induced DDR and, therefore, may cause genomic instability against radiation in epithelial stem cells through its transcriptional repressive activity on p53, consequently, contributing to radiation-induced carcinogenesis.

Funding Source: This research was supported by the Japan Society for the Promotion of Science (JSPS) (grant no. JP19K20455) and Network-type Joint Usage/Research Center for Radiation Disaster Medical Science (grant no. T21-01-011).
Keywords: ΔNp63α, DNA damage response, Anti-apoptosis

TOPIC: CARDIAC

104

CHALLENGING TRADITIONAL CELL FATE DECISIONS DURING CORONARY ARTERY DEVELOPMENT

Rios Coronado, Pamela E - *Biology, Stanford University, Stanford, CA, USA*
Fan, Irene - *Biology, Stanford University, Stanford, CA, USA*
Gonzalez, Karen - *Stem Cell and Regenerative Medicine Institute, Stanford University, Stanford, CA, USA*
Sy-Quia, Ana - *Biology, Stanford University, Stanford, CA, USA*

Abstract: Coronary artery disease (CAD) is the leading cause of death worldwide, resulting from vascular blockages that reduce blood flow to cardiac muscle. Investigating how the endothelial cells (EC) that line blood vessels are assembled into functional coronary arteries could identify revascularization strategies that treat CAD. The coronary vascular bed is comprised of arteries, capillaries, and veins. Our lab demonstrated that coronary arteries develop during embryogenesis when capillary plexus ECs exit the cell cycle and differentiate into artery ECs that subsequently migrate together to form a mature artery. However, a subset of artery ECs are left behind during this process and revert to a capillary EC fate. This ultimately creates a capillary bed comprised of cells that experienced two different life histories: those with a linear path to the capillary fate and those that differentiated into arteries and then back into capillaries—a reverted path. Our preliminary observations during embryonic and neonatal development, show that reverted path cells share similar proliferation and clonal expansion patterns with arterial ECs rather than the neighboring capillaries ECs. In accordance with evidence that cell cycle exit and suppression potentiates arterial specification, we hypothesize that they are primed to contribute to the massive expansion of coronary arteries that occurs during postnatal heart growth and in response to injury. We first, used tissue clearing/whole organ imaging to detail the extensive expansion of coronary arteries after birth and into adult stages to pin-point critical anatomical changes and developmental trajectories that produce coronary arteries during postnatal heart growth. Next, we used single-cell RNA sequencing to gain evidence that reverted path ECs are primed. The existence of the reversion events presents the important question of whether the developmental heterogeneity of capillary ECs is crucial in their ability to react to regenerative and post-birth developmental signals.

Keywords: coronary artery, endothelial cell, cardiovascular

TOPIC: EARLY EMBRYO

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SPATIOTEMPORAL LINEAGE DEREPRESSION BY ETV4 INACTIVATION IN HUMAN EMBRYONIC STEM CELLS

Yang, Seungbok - *Lifescience, POSTECH, Pohang, Korea*
Oh, Yujeong - *Lifescience, POSTECH, Pohang, Korea*
Oh, Seyoun - *Lifescience, POSTECH, Pohang, Korea*
Cho, Yoonjae - *Lifescience, POSTECH, Pohang, Korea*
Yoe, Jeehyun - *Lifescience, POSTECH, Pohang, Korea*
Lee, Yoontae - *Lifescience, POSTECH, Pohang, Korea*
Jang, Jiwon - *Lifescience, POSTECH, Pohang, Korea*

Abstract: One of the key questions in developmental biology is how multi-lineage derivation arises from a seemingly identical stem cell population. Self-organization is a cellular process that creates multiple cell fates without specific prepatterns during embryonic development. Despite the importance of self-organization, the mechanism is unclear. Using In vitro hESC culture as an experimental platform, we discovered differences in spatiotemporal differentiation potential in hESC colonies. Neuroectoderm is initially repressed in small hESC colonies, but as the colony size increases, center cells acquire neuroectoderm potential. Mechanistically, we found that ETV4 is a master repressor of the neuroectoderm and ETV4 inactivation in the center of large colonies is essential for neuroectoderm derepression. Because ETV4 inactivation occurs prior to differentiation, ETV4 serves as an initial symmetry breaker that determines spatiotemporal regulation of lineage fates. Taken together, these results suggest that derepression is a critical mechanism of NE derivation from hESCs with ETV4 positioned as a key regulator. Moreover, ETV4 inactivation in large hESC colonies is a critical cellular process underlying multi-lineage derivation.

Keywords: human embryonic stem cell, self-organization, ETV4

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PLURIPOTENCY AND IMPLANTATION: UNRAVELLING THE COUPLING OF METABOLISM TO CHROMATIN STATES

Pladevall-Morera, David - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Frater, Ciaran - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Bages-Arnal, Sandra - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Casòliba-Melich, Arnau - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Argemi-Muntadas, Lidia - *Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark*
Knudsen, Teresa E. - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Brickman, Joshua M. - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Moritz, Thomas - *Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark*
Zylicz, Jan - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*

Abstract: Embryo implantation is the stage of pregnancy in which the embryo invades the uterus and starts to grow rapidly. Yet this

critical moment is also the most common stage when pregnancy fails. On the molecular level implantation is associated with a cellular metabolic and epigenetic reprogramming. While it is known that rapid changes to chromatin modifications coordinate early development, precisely how these dynamic processes are regulated is unclear. Interestingly, metabolism, and in particular some intermediates of metabolic reactions exert a non-canonical function by acting as co-factors and substrates of chromatin modifying enzymes. Therefore, fluctuating availability of such molecules has the potential to regulate epigenetic landscape and cell fate. Here we investigate how this metabolic reprogramming is functionally linked to chromatin changes and transcriptional transitions to allow successful implantation. To address this challenge, we will combine multiple –omics strategies, including metabolomics, epigenomics and transcriptomics, with functional assays in vivo and in vitro. By acutely depleting specific metabolic enzymes in mouse embryonic stem cells we alter the metabolic state of cells and study the effects on the epigenetic landscape and cell state transitions. This investigation will shed light on the molecular mechanisms that define the interplay between metabolism and epigenetics in the early stages of development. In addition, it should help to understand how environmental factors influence embryo implantation.

Funding Source: This research is supported by The Novo Nordisk Foundation renew grant number NNF21CC0073729; Danmarks Frie Forskingsfond grant number 0169-00031B; and Lundbeck Fonden grant number R345-2020-1497.
Keywords: metabolism, epigenetics, implantation

TOPIC: EPITHELIAL_GUT

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HUMAN SALIVARY GLAND ORGANOIDS DISPLAY LONG-TERM GENETIC STABILITY AND INTACT FUNCTIONALITY WITH DIVERSE CELLULAR HETEROGENEITY

Kim, Donghyun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul*
Yoon, Yeo-Jun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Tak, Kwon Yong - *Graduate School of Medical Science and Engineering, Korean Advanced Institute of Science and Technology, Daejeon, Korea*
Lee, Soohyun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Hwang, Seungyeon - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Kim, Jisun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Sim, Nam Suk - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Cho, Jae-Min - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Choi, Dojin - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Kim, Hyunki - *Pathology, Yonsei University College of Medicine, Seoul, Korea*
Park, Jong-Eun - *Graduate School of Medical Science and Engineering, Korean Advanced Institute of Science and Technology, Daejeon, Korea*
Lim, Jae-Yol - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*

Abstract: 3D in vitro organoid culture enables maintenance and expansion of physiological tissue-like cellular cluster from adult stem cells. For clinical application and exploitation of organoids, long-term maintenance of organoids with consistent gene expression, no genetic alteration, and intact functionality is prerequisite. Although some advances in salivary gland stem cells have brought the development of 3D organoid culture derived from both murine and human salivary glands, the capability of long-term culture have barely been addressed. In this study, we successfully maintained human salivary gland organoids up to 4 months without obvious morphological changes. Furthermore, gene expression analysis indicated that ductal, acinar, and myoepithelial cell markers were consistently expressed during organoid culture. Glandular functions were assessed via neurotransmitter-induced calcium influx and organoid swelling assay, resulting in comparable functionality between short- and long-term maintained organoids. In addition, cellular composition determined by RNAseq at single cell resolution further revealed maintenance of diverse subpopulation during long-term culture. Altogether, these results suggest that our culture system has a potential for long-term exploitation of salivary gland organoids and platform for drug screening or diagnostic test.

Keywords: Salivary gland organoids, scRNAseq, long-term culture

TOPIC: GERMLINE

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TEN ELEVEN TRANSLOCATION 1 (TET1) IS REQUIRED FOR HUMAN PRIMORDIAL GERM CELL-LIKE CELL SPECIFICATION FROM PLURIPOTENT STEM CELLS

Hsu, Fei-Man - *MCDB, University of California, Los Angeles, CA, USA*
Wu, Qiu-Ya - *MCDB, University of California, Los Angeles, CA, USA*
Fabyanic, Emily - *Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
Wu, Hao - *Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, USA*
Clark, Amander - *Molecular, Cell & Developmental Biology, University of California, Los Angeles, CA, USA*

Abstract: The mammalian germline undergoes waves of DNA methylation remodeling resulting in global loss of DNA methylation in the pre-implantation embryo and primordial germ cells (PGCs) with global gains of DNA methylation during embryo implantation and with the formation of gametes. Using the mouse as a model, removal of DNA methylation from germline DNA involves oxidation of 5mC (5-methylcytosine) to 5hmC (5-hydroxymethylcytosine) by the Ten eleven translocation (TET) enzyme TET1 in PGCs, and TET3 in pre-implantation embryos. In mouse PGCs, the function of TET1/5hmC occurs at the end of PGC development, whereas in other mammals (porcine and non-human primates) TET1/5hmC is enriched at the time of PGC specification soon after embryo implantation. Given these species-specific differences in the timing of 5hmC accumulation, and the challenges in studying early post-implantation human embryo development, we used human embryonic stem cells (hESCs) and the differentiated human PGC-like cells (hPGCLCs) to model hPGC specification in vitro. Using APOBEC Coupled Epigenetic Sequencing (ACE-Seq), we discovered that the specification of hPGCLCs is

associated with a statistically significant increase in 5hmC in the hPGCLC genome comparing to the undifferentiated hESCs. To identify the TET protein responsible for this 5hmC enrichment we used single cell RNA-Seq and immunofluorescent staining and show TET1 RNA and protein are expressed by hPGCLCs, whereas TET2 and TET3 RNAs are undetectable. Given this, we hypothesized that TET1/5hmC functions at the time of hPGC specification to regulate for formation of hPGCLCs. To address this, we created multiple TET1 catalytic domain CRISPR knockout (CDKO) hESC sublines. Although mild effects on hESC gene expression in CDKO sublines were shown in RNAseq, the 5hmC levels in the CDKO undifferentiated hESCs were drastically reduced. By differentiating the CDKO and control hESC sublines into hPGCLCs, we show that hPGCLC specification is significantly reduced in the CDKO lines. However once specified, the CDKO hPGCLCs can be maintained in extended culture. Taken together, our work shows that TET1 catalytic domain activity is required for hPGCLC specification but not for early hPGCLC maintenance once specification is complete.

Funding Source: Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA training program
Keywords: Primordial germ cell, Ten-eleven translocation 1(TET1), 5-hydroxymethylcytosine (5hmC)

TOPIC: HEMATOPOIETIC SYSTEM

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METABOLIC REGULATION OF BLOOD-PROGENITOR DEVELOPMENT IN DROSOPHILA

Goyal, Manisha - *Regulation of Cell Fate, Institute for Stem Cell Science and Regenerative Medicine (inStem), Bengaluru, India*
Tomar, Ajay - *Regulation of Cell Fate, Institute For Stem Cell Science and Regenerative Medicine (inStem), Bengaluru, India*
Madhwal, Sukanya - *Regulation of Cell Fate, Institute For Stem Cell Science and Regenerative Medicine (inStem), Bengaluru, India*
Mukherjee, Tina - *Regulation of Cell Fate, Institute For Stem Cell Science and Regenerative Medicine (inStem), Bengaluru, India*

Abstract: Akin to mammalian myeloid development, Drosophila hematopoiesis takes place in two different waves, where the second and definitive wave takes place in a specialized larval hematopoietic organ termed the lymph gland, which is divided into various compartments. It harbours blood-progenitors and differentiated cells in the medullary zone and cortical zone respectively, and an intermediate zone comprises the differentiating progenitor cells. Various signaling cues have been shown to regulate lymph gland development, and recent studies shed light on the involvement of metabolic pathways in myeloid development. Among these metabolites, the role of reactive oxygen species (ROS) in myeloid development is well established and its aberrant generation alters hematopoiesis. Thus, maintaining homeostatic levels of ROS is very crucial for the blood-progenitor cells. Any understanding of intracellular metabolic or signaling events that enable the sustenance of this fine redox balance and blood-progenitor development remains obscure. We show that, in homeostasis, myeloid-like blood-progenitor cells of the Drosophila larvae utilize the TCA cycle to generate ROS. However, excessive ROS production leads to lymph gland growth retardation. Therefore, to moderate blood-progenitor ROS, Drosophila larvae rely on olfaction and its downstream systemic GABA. Further, GABA catabolism controls antioxidant synthesis necessary to scavenge any excess ROS that is generated. We have identified the metabolic requirement of odor sensing and GABA in regulating redox homeostasis during Drosophila myeloid progenitor development, the relevance of which may be broadly conserved.

Keywords: GABA Metabolism, Reactive Oxygen Species, Blood-progenitors

TOPIC: KIDNEY

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DIVERSITY-GENERATING MECHANISMS FOR MAMMALIAN INTERCALATED CELL LINEAGES

Parvez, Riana K. - *Development, Stem Cell, and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
McMahon, Andrew - *Development, Stem Cell, and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Three-quarters of a million Americans have End Stage Renal Disease (ESRD). Their only treatment options are dialysis, with poor long term survival prospects, or a curative kidney transplant, with too few kidneys to meet patient need. One way to address the shortage of transplantable kidneys is to develop a thorough insight into essential cell types and how they are generated, and use this insight to generate functional, cell-based kidney replacements. The functional epithelial networks of the kidney arise from two distinct progenitor populations. Nephron progenitors generate approximately 1 million nephrons in the human kidney, and adjacent ureteric progenitors generate the branched network of the collecting duct, to which nephrons connect. Homeostasis is maintained by specialized cell types in both the nephron and the collecting duct. Water/salt and pH balance, are regulated by principal cells and intercalated cells (ICs), respectively. There are 3 subtypes of ICs: type A intercalated cells (A-ICs), type B intercalated cells (B-ICs) and non-A-non-B intercalated cells that perform distinct functions in the regulation of pH homeostasis. Recently, the McMahon lab demonstrated both PCs and ICs have dual origins from both nephron and ureteric progenitor cells, an unusual developmental process. We aim to identify the mechanisms generating similar, but not identical, IC subtypes from distinct kidney progenitor populations. We identified several transcriptional regulators that we predict are involved in IC subtype development. Dmrt2 distinguishes both nephron and ureteric epithelial derived A-IC cell types from Hmx2/Hmx3 expressing IC-B cell types. Differential Hmx gene expression sub-divides the B-IC populations: Hmx2+ B-ICs and non-A-non-B ICs are nephron-derived and Hmx2+/Hmx3+ B-ICs are ureteric lineage derived. We hypothesize that Tfcp2l1 and Foxi1 initiate a general IC developmental program, while Dmrt2, Hmx2, and Hmx3 have mutually repressive actions in the generation of IC subtypes. We have generated knockout models for Dmrt2 and Hmx3; Hmx2. We observe that a loss of Dmrt2 results in an expansion of Hmx2 expression, suggesting a repressive role for Dmrt2 in A-ICs. Taken together, this work provides new insights into the regulatory programs governing cell types involved in mammalian kidney function.

Funding Source: ASN Kidney Cure Pre-Doctoral Fellowship
Keywords: kidney, development, cell fate

TOPIC: MUSCULOSKELETAL

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SINGLE-CELL RNA-SEQUENCING OF PATIENT-DERIVED PRIMARY CELLS DISSECTS THE MOLECULAR OSTEOGENIC DIFFERENTIATION DEFECTS CAUSING A SOMATIC SKELETAL DISEASE

Paria, Nandina - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Khalid, Aysha - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Lemoine, Benjamin - *Baylor Scott & White Research Institute, Dallas, TX, USA*
Chan, Jinyan - *Scottish Rite for Children, Waco, TX, USA*
Kidane, Yared - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Oxendine, Ila - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Cornelia, Reuel - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Wise, Carol - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*
Rios, Jonathan - *Center for Pediatric Bone Biology and Translational Research, Scottish Rite for Children, Dallas, TX, USA*

Abstract: Neurofibromatosis Type 1 (NF1) is a tumor predisposition syndrome that may also include multiple skeletal manifestations, including fracture healing defects (pseudarthroses) that are associated with somatic mutations in the NF1 gene. To elucidate the molecular consequence of somatic NF1 loss (NF1-/-) on mesenchymal stromal cell (MSC) osteogenesis, we performed time-series single-cell RNA-sequencing (scRNAseq) and trajectory analyses throughout osteogenic differentiation using primary MSCs cultured from matched patient samples of unaffected control and pseudarthrosis bone. Using gene set enrichment analysis (GSEA), control bone MSC (NF1+/) trajectory pseudotime was associated with increased expression of genes involved in osteoblast differentiation. We next performed time-series scRNAseq using fracture-derived MSCs, which consist of NF1+/- and somatic NF1-/- MSCs. NF1-/- MSCs express high levels of EREG, encoding Epiregulin. We detected a minority EREGHIGH cell populations at all timepoints, with the majority of cells being EREGLOW (NF1+/-). EREGLOW pseudotime was associated with increased expression of genes involved in osteoblast differentiation, similar to pseudotime analysis of control bone-derived MSCs. These results suggest osteogenic differentiation of NF1+/- MSCs is not negatively affected by co-culture with NF1-/- MSCs. In contrast, EREGHIGH pseudotime was associated with increased expression of genes negatively regulating osteoblast differentiation, despite showing increased expression of pro-osteogenic genes. We then tested osteogenic differentiation in fracture-derived MScs by single-cell qPCR which showed increased expression of RUNX2 in both NF1+/- and NF1-/- cells. We also detected EREG+ bone-lining osteoblasts in patient fracture tissue by immunohistochemistry. Further, scRNAseq revealed significantly reduced global expression of genes involved in extracellular matrix (ECM) formation and mineralization in NF1-/- cells. Our results show that cells taken from NF1 pseudoarthroses are associated with osteoblast dysfunctions that include ECM mineralization, but not differentiation. Our application of scRNAseq to primary, mixed-population patient cells should prove useful for exploring the cellular mechanisms of somatic skeletal disease.

Funding Source: Pediatric Orthopaedic Society of North America, Texas Neurofibromatosis Foundation, Department of Defense, National Cancer Institute
Keywords: Single-cell RNA sequencing, Mesenchymal stromal cell, Neurofibromatosis

TOPIC: NEURAL

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USING HUMAN PLURIPOTENT STEM CELLS TO UNDERSTAND AND MODEL SPECIFICATION OF THE HUMAN GANGLIONIC EMINENCES

Hunt, Cameron - *Stem Cells and Neural Development, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*
Gantner, Carlos - *Department of Physiology, Development and Neuroscience, University of Cambridge, UK*
Moriarty, Niamh - *Stem Cells and Neural Development, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*
Parish, Clare - *Stem Cells and Neural Development, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*
Thompson, Lachlan - *Neurogenesis and Neural Transplantation Laboratory, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*
Van Deursen, Coen - *Stem Cells and Neural Development, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*

Abstract: GABA producing cortical interneurons integrate with excitatory cortical projection neurons in sensory and motor cortices to form functioning circuitries. Developmentally, these critical neural subtypes are generated from three major zones in the ventral forebrain (subpallium); either the lateral-, medial- or caudal ganglionic eminences (GE) but migrate dorsally to reside in within layers of the cortex. Interestingly, despite the different layers and cortical regions these interneurons integrate into, these developmental eminences lie close in proximity to one another in the subpallium with progenitors across different zones sharing a significant portion of their molecular profile. A distinct neurochemical profile only emerges following migration and further differentiation. The degeneration of these cortical circuitries is central to several neurological disorders including motor neuron disease and frontotemporal dementia. Therefore, to develop more accurate and physiologically relevant disease models or cell transplantation therapies from human pluripotent stem cell (hPSC)-derived cortical cell types, understanding the highly nuanced development of these sub pallial progenitors is crucial. Here we utilize hPSC reporter lines (NKX2.1-GFP and MEIS2-mCherry) together with directed differentiation protocols to gain greater insight into the spatiotemporal roles of early sonic hedgehog (SHH), WNT and retinoic acid (RA) signalling in the regional specification of the GE zones. We identified the role of SHH-WNT signalling cross-talk in regulating LGE and MGE fate decisions and uncovered a novel role for RA signalling in CGE-derived interneurons. Unravelling the influence of these signalling moieties permitted the development of fully-defined, directed-differentiation protocols for hPSCs that favoured the generation of progenitors from the three GE domains. hPSC reporter lines enabled further population enrich-

ment, that subsequently matured & integrated into defined neuronal populations following transplantation. These findings provide insight into the context-dependent role of key morphogens in the specification of the human ventral forebrain and will be a valuable resource to profile hPSC-derived subpallial cell types for disease modelling and advancement of new therapies.

Keywords: Neural differentiation, Human ganglionic eminence, In vitro specification of hiPSCs

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DEVELOPMENT OF A RAPID AND EFFICIENT DIFFERENTIATION PROTOCOL FOR THE GENERATION OF FUNCTIONAL ASTROCYTES FROM HUMAN IPSCS

Kilander, Michaela - *Elixirgen Scientific, Baltimore, MD, USA*
Ahmad, Muzammil - *Elixirgen Scientific, Baltimore, MD, USA*
Sharov, Alexei - *Elixirgen Scientific, Baltimore, MD, USA*
Samra, Amandeep - *Elixirgen Scientific, Baltimore, MD, USA*
Fraher, Brianna - *Elixirgen Scientific, Baltimore, MD, USA*
Jeffries, Emiko - *Elixirgen Scientific, Baltimore, MD, USA*
Ko, Minoru - *Elixirgen Scientific, Baltimore, MD, USA*

Abstract: Astrocytes are the most abundant glial cell type in the human central nervous system (CNS). They play crucial roles in human neurophysiology by participating in neurotransmission; supporting neuronal metabolic processes; regulating extracellular pH and levels of K+, GABA and glutamate; modulating tissue injury response and repair; controlling cerebral blood flow; and maintaining the integrity of the blood brain barrier. Dysfunctional astrocytes have been implicated in neurodevelopmental conditions and neurodegenerative disorders such as epilepsy, amyotrophic lateral sclerosis, autism spectrum disorders, schizophrenia, Huntington's disease, Alzheimer's disease, and Parkinson's disease. As human primary astrocytes are difficult to obtain, induced pluripotent stem cells (iPSCs) provide a unique opportunity to study glial physiology and pathophysiology in humans, but progress has been hampered due to the difficult and time-consuming process of generating functional astrocytes from iPSCs. Using Elixirgen Scientific's proprietary transcription factor-based Quick-Tissue™ technology, we here describe a rapid differentiation protocol which can efficiently produce functional astrocytes from iPSCs in only 42 days. Transcriptome analysis by RNA-seq and RT-qPCR using human primary astrocytes as a reference, demonstrated a progressive development of iPSCs towards astrocyte-like cells. We subsequently established the presence of several astrocyte marker proteins, such as ALDH1L1, GFAP, CD44, CD49f, and S100β by immunocytochemical detection. Furthermore, we found that these cells are able to clear glutamate, a hallmark of the astrocyte functional phenotype, in a manner similar to that of human primary astrocytes. In conclusion, we have established a protocol for fast, reliable, and robust differentiation of iPSCs to astrocyte-like cells. Our Quick-Tissue™ technology yields reproducible results and has been successfully applied to iPSC lines derived from both healthy and diseased donors, thus providing researchers with useful tools to make new discoveries in the roles of astrocytes in brain development, aging, and disorders.

Keywords: IPSC DIFFERENTIATION, ASTROCYTES, TRANSCRIPTION FACTORS

TOPIC: PANCREAS

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PROTOCOL DEVELOPMENT TO FURTHER DIFFERENTIATE AND TRANSITION HPSC-DERIVED PANCREATIC PROGENITORS FROM A MONOLAYER INTO ENDOCRINE CELLS EITHER IN SUSPENSION CULTURE OR FOLLOWING IMPLANTATION INTO MICE

Braam, Mitchell J.S. - *Department of Cellular & Physiological Sciences, The University of British Colombia, North Vancouver, BC, Canada*
Ida, Shogo - *Department of Cellular and Physiological Sciences, The University of British Colombia, Vancouver, BC, Canada*
Baker, Robert - *Department of Cellular and Physiological Sciences, The University of British Colombia, Vancouver, BC, Canada*
Kloostra, Nick - *Department of Cellular and Physiological Sciences, The University of British Colombia, Vancouver, BC, Canada*
Tang, Mei - *Department of Cellular and Physiological Sciences, The University of British Colombia, Vancouver, BC, Canada*
Quiskamp, Nina - *STEMCELL Technologies Inc., Vancouver, BC, Canada*
Piret, James - *Department of Chemical and Biological Engineering, The University of British Colombia, Vancouver, BC, Canada*
Kieffer, Timothy - *Department of Cellular and Physiological Sciences, The University of British Colombia, Vancouver, BC, Canada*

Abstract: The generation of functional beta-cells from human pluripotent stem cells (hPSCs) for cell replacement therapy and disease modeling of diabetes is being investigated by many groups. Recent scientific breakthroughs have enabled derivation of large quantities of human pancreatic beta-like cells in the laboratory, but current protocol yields can be variable, especially for cells of different genetic backgrounds. The STEMdiff™ Pancreatic Progenitor kit provides serum-free, defined media that supports efficient and reproducible generation of pancreatic progenitor cells from mTeSR™1-maintained hPSCs in a 2D monolayer. We have developed a protocol to harvest and aggregate kit-derived pancreatic progenitors from several different cell lines into uniform spheroids and to further differentiate the cells toward an endocrine-cell fate using suspension culture. Aggregate yield was an important parameter that we focused on, as early iterations of the protocol caused a considerable amount of cell loss. By comparing several previously reported endocrine progenitor medium formulations, we identified FGF7 as a promoter of aggregate survival during endocrine differentiation in suspension culture, though with a decrease in endocrine gene expression. We next tested suppression of SOX9 expression and induction of NGN3 expression during cell aggregation. We found that the treatment of pancreatic progenitor cells with the Notch-inhibitor DAPT during aggregation improved endocrine cell induction in vitro and the cells secreted more human C-peptide following cell implantation and maturation in mice. Our most recently optimized protocol improves aggregate survival in suspension culture and initiates NGN3 induction and SOX9 reduction as cells progress toward the endocrine lineage. Future work will focus on the in

vitro maturation of the generated endocrine cells to improve their function.

Funding Source: Funding for this research was provided by Mitacs, Canadian Institutes of Health Research, Juvenile Diabetes Research Foundation, National Institutes of Health, and Canada Foundation for Innovation.
Keywords: Differentiation, Pancreas, Diabetes

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DECODING SINGLE-CELL GENE REGULATORY NETWORKS OF THE HUMAN ADULT PANCREAS

Vanheer, Lotte - *Development and Regeneration, Katholieke Universiteit Leuven, Belgium*
Fantuzzi, Federica - *ULB Center of Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium*
To, San Kit - *Department of Development and Regeneration, KU Leuven, Belgium*
Schiavo, Andrea - *ULB Center of Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium*
Van Haele, Matthias - *Department of Imaging and Pathology, KU Leuven, Belgium*
Haesen, Tine - *Department of Development and Regeneration, KU Leuven, Belgium*
Janiszewski, Adrian - *Department of Development and Regeneration, KU Leuven, Belgium*
Chappell, Joel - *Department of Development and Regeneration, KU Leuven, Belgium*
Rihoux, Adrien - *Department of Development and Regeneration, KU Leuven, Belgium*
Roskams, Tania - *Department of Imaging and Pathology, KU Leuven, Belgium*
Cnop, Miriam - *ULB Center of Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium*
Pasque, Vincent - *Department of Development and Regeneration, KU Leuven, Belgium*

Abstract: Transcription factors recognize and interact with cis-regulatory elements, forming gene regulatory networks, that together with chromatin modifications and topology, alter gene expression and determine cell fate. While important insights into pancreatic cellular identity have been obtained using single cell transcriptomics, the underlying gene regulatory logic remains poorly understood. Here, we integrate multiple, publicly available, single-cell RNA sequencing datasets of the human adult pancreas to provide an integrated cell atlas of 7393 single cells of human adult pancreata from healthy and type 2 diabetic individuals. We then infer gene regulatory networks using single-cell regulatory network inference and clustering (SCENIC). We present evidence that our approach identified key regulators of cell identity in the human adult pancreas. Specifically, we predict that HEYL, BHLHE41 and JUND are active in acinar, beta and alpha cells, respectively, and show that these proteins are present in the human adult pancreas as well as in human induced pluripotent stem cell-derived pancreatic cells. This comprehensive gene regulatory network atlas can be explored interactively online. These findings provide a first in-depth look at the gene regulatory logic underlying cellular identity in the human adult pancreas in a broad range of healthy and type 2 diabetic individuals. We anticipate our analysis to be the starting point for a more sophisticated dissection of how transcription factors regulate cell identity in the human adult pancreas.

Funding Source: This work is supported by the FWO, KU Leuven, FNRS, Brussels Region Innoviris project DiaType, Win2Wal program of the Walloon Region, INNODIA, EFPIA, JDRF and The Leona M. and Harry B. Helmsley Charitable Trust.
Keywords: Human Pancreas, Transcription Factors, Gene Regulatory Networks

TOPIC: PLURIPOTENT STEM CELLS

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ZBTB12 ACTS AS A MOLECULAR BARRIER FOR DEDIFFERENTIATION BY SUPPRESSING HERVH-DERIVED LNCRNAS IN HUMAN PLURIPOTENT STEM CELLS

Oh, Yujeong - *Department of Life Sciences, POSTECH, Pohang, Korea*
Han, Dasol - *Department of Molecular, Cellular, and Developmental Biology, Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Liu, Guojing - *Department of Molecular, Cellular, and Developmental Biology, Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Oh, Seyoun - *Department of Life Sciences, POSTECH, Pohang, Korea*
Yang, Seungbok - *Department of Life Sciences, POSTECH, Pohang, Korea*
Rani, Neha - *Indian Institute of Technology, Department of Biological Sciences & Bioengineering, Kanpur, India*
Almeida, Maria C. - *Department of Biological Sciences, Federal University of ABC, Center for Natural and Human Sciences São Bernardo do Campo, Santo André, Brazil*
Kosik, Kenneth S. - *Department of Molecular, Cellular, and Developmental Biology, Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Jang, Jiwon - *Department of Life Sciences, POSTECH, Pohang, Korea*

Abstract: Development or stem cell differentiation is generally viewed as one-way traffic of cell state transition from primitive to developmentally advanced states. However, molecular mechanisms that ensure the unidirectional transition of cell fates remain largely unknown. Through exact transcription start site mapping and transcription factor motif analysis, we identified an evolutionarily conserved BTB domain-containing zinc finger protein, ZBTB12, as a molecular barrier for dedifferentiation of human pluripotent stem cells (hPSCs). Single cell RNA sequencing revealed that ZBTB12 was essential for three germ layer differentiation by blocking dedifferentiation of hPSCs toward a more primitive state. Mechanistically, ZBTB12 serves as a master repressor of human endogenous retrovirus H (HERVH), a primate-specific retrotransposon. ZBTB12-mediated suppression drives efficient shutdown of young and transcriptionally active HERVH loci upon pluripotency exit. Active HERVH loci act as strong promoters for overlapping long non-coding RNAs (lncRNAs) whose downregulation by ZBTB12 is necessary for successful exit from a pluripotent state and lineage derivation. Overall, we have identified ZBTB12 HERVH-derived lncRNAs is a molecular machinery that safeguards the unidirectional transition of metastable stem cell fates toward developmentally advanced states.

Keywords: Human pluripotent stem cells, Unidirectional differentiation, Retrotransposons

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UNRAVELING GENETIC MOSAICISM: FROM HUMAN EMBRYONIC STEM CELLS TO RETINAL PIGMENT EPITHELIUM CELLS

Couvreu de Deckersberg, Edouard - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Regin, Marius - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Scheyltjens, Isabelle - *Laboratory of Molecular and Cellular Therapy, Vrije Universiteit Brussel, Belgium*
Tsuiko, Olga - *Laboratorium voor Cytogenetica en Genoomonderzoek, Katholieke Universiteit Leuven, Belgium*
Le, Yingan - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Krivec, Nuša - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Sermon, Karen - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*
Spits, Claudia - *Reproduction and Genetics, Vrije Universiteit Brussel, Belgium*

Abstract: The genomic instability of hPSC, reminiscent of cancer cells is a safety concern for their clinical translation. In this study we investigate the evolution of genetic mosaicism upon differentiation of hESC to retinal pigment epithelium cells (RPE), a cell type broadly used in clinical trials. We aim at establishing if and which copy number variants (CNV) can arise or enrich during differentiation and how they affect RPE gene expression. We differentiated 5 genetically balanced hESC lines (established by bulk shallow whole genome sequencing) to RPE. We performed single-cell DNA and RNA sequencing (scDNAseq & scRNAseq) of 3 hESC lines at the undifferentiated state (VUB02, VUB04, VUB07) and 5 hESC-derived RPE cell cultures (VUB02, VUB04, VUB07, VUB14, VUB32), respectively. InferCNV on the scRNAseq data was used to predict the CNV present in the RPE. hESC' scDNA-seq showed that 2.5% of cells in all 3 lines had complex karyotypes with multiple monosomies and trisomies, and 5%, 8.3% and 12% of cells in VUB02, VUB04 and VUB07 carried CNV >10Mb. 3.3% of cells of VUB04 carried a dup1q and 2% of cells in VUB07 had a large gain of 20q11.21; both are known recurrent CNV. InferCNV analysis of the scRNAseq (#cells range from 15916 to 22530) showed that dup1q were present in all five RPE samples. The two lines in which we found no dup1q in the scDNAseq had 5% and 9% of dup1q cells in RPE, while VUB04, with 3% of dup1q in the scDNAseq, ended with 54% of dup1q cells in RPE. We also found a variety of other CNV in all 5 RPE samples, mainly duplications. Unlike in hESC, we never found full monosomy or trisomy in RPE, the CNV size ranging 1.2-78Mb. There also was line-to-line differences, with variant cells representing 20% to 85% of the population. We are now validating these findings by ddPCR. The results for the gain of 20q11.21 show that 2 of 5 hESC carried it at 2% and 11%, the first did not progress and the other one to 36% after RPE differentiation. Finally, transcriptome analysis of the scRNA-seq shows a recurrent heterogeneity in the RPE. While all the cells express RPE genes, we also found cells exhibiting profiles of neural progenitor cells, immunity-like or cells expressing tumor suppressor genes. The next step of our study will be to link the transcriptomic profile to the aneuploidies found in the cells.

Funding Source: FWO PhD fellowship strategic basic research
Keywords: human embryonic stem cells, retinal pigment epithelium, mosaicism

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TASOR IS ESSENTIAL FOR NORMAL H3K9ME3 HETEROCHROMATIN ESTABLISHMENT DURING EARLY MAMMALIAN DEVELOPMENT

Pinzon-Arteaga, Carlos A. - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
O'Hara, Ryan - *Department of Obstetrics and Gynecology, UT Southwestern Medical Center, Dallas, TX, USA*
Sakurai, Masahiro - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Wei, Yulei - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Schmitz, Daniel - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Pawłowski, Krzysztof - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Tagliabracci, Vincent - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Banaszynski, Laura - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Wu, Jun - *Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*

Abstract: Heterochromatin is a highly condensed form of chromatin associated with transcriptional repression and is essential for normal development and genomic stability. Heterochromatin, often marked by the trimethylation of lysine 9 of histone H3 (H3K9me3) and 5-methylcytosine (5mC) CpG methylation, is typically found at repetitive elements and constitutes a major epigenetic barrier of cell fate changes during development and nuclear reprograming. During early embryonic development, the genome undergoes a series of essential epigenetic remodeling events, including the global erasure and eventual re-establishment of 5mC. Coinciding with these events are the highly-coordinated up-regulation of numerous repetitive element families. How the pluripotent genome safeguards itself from unchecked activation of repetitive elements during global demethylation and the mechanisms which re-establish heterochromatin at later developmental stages remain poorly understood. We found that FAM208a (also known as transgene activator suppressor, or TASOR and part of the human silencing hub (HUSH) complex) is required for proper H3K9me3 deposition at LINE-1 transposable elements, major satellite, centromeric and telomeric repeats in naïve mouse embryonic stem cells (mESCs). TASORKO mESCs showed reduced proliferation, defects in 5mC methylation, upregulation of LINE-1 elements and expression of chimeric transcripts originating from LINE-1 promoters, and exhibited massive cell death upon transition to formative epiblast-like cells (EpiLCs), or formative stem cells (FS). Interestingly, TASOR is not expressed in EpiSCs, suggesting that EpiSCs survival is dependent on TASOR-mediated H3K9me3 deposition during the preceding naïve state.

Funding Source: The Welch Foundation 854671, NIH R01 GM138565-01A1
Keywords: Heterochromatin, H3K9me3, Stem cells

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POLYCOMB REPRESSIVE COMPLEX 2 RESTRICTS HUMAN TROPHOBLAST INDUCTION

Talon, Irene - *Development and Regeneration, Katholieke Universiteit Leuven, Belgium*
Zijlmans, Dick - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*
Verhelst, Sigrid - *ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium*
Bendall, Adam - *Epigenetics Programme, Babraham Institute, Cambridge, UK*
Van Nerum, Karlien - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Javali, Alok - *Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria*
Malcolm, Andrew - *Epigenetics Programme, Babraham Institute, Cambridge, UK*
van Knippenberg, Sam - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
To, San Kit - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Janiszewski, Adrian - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Corthout, Nikky - *VIB Center for Brain & Disease Research, University of Leuven (KU Leuven), Belgium*
Balaton, Bradley - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Georgolopoulos, Grigorios - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Panda, Amitesh - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Allsop, Ryan - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Chappell, Joel - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Pham, Thi Xuan Ai - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Oberhuemer, Michael - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Vanheer, Lotte - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*
Rivron, Nicolas - *Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria*
Dhaenens, Maarten - *ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Leuven, Belgium*
Marks, Hendrik - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*
Rugg-Gunn, Peter - *Epigenetics Programme, Babraham Institute, Cambridge, UK*
Pasque, Vincent - *Department of Development and Regeneration, University of Leuven (KU Leuven), Belgium*

Abstract: During human development, the first cell fate specification event leads to the distinction between the extraembryonic trophectoderm cells and the embryonic pluripotent epiblast. However, lineage commitment is not complete in the early blastocyst. This unrestricted lineage potential is retained in vitro in naive human pluripotent stem cells (hPSCs), which can give rise to embryonic and extraembryonic lineages, and are able to form blastoids resembling in vivo human blastocysts. Yet, it is currently unknown which chromatin mechanisms enable the developmental plasticity present in naive hPSCs. Here, we show that the Polycomb Repressive Complex 2 (PRC2) acts as a barrier to alternative cell fates in the human naive state and in human blastoids. First, we detected the presence of PRC2-mediated H3K27me3 at the promoters of key lineage regulators in naive hPSCs, including trophoblast regulators, suggesting that PRC2 might oppose cell fate specification in naive hPSCs. Next, we inhibited PRC2 during naive to trophoblast conversion. We found that PRC2 inhibition (PRC2i) increased the number of GATA3-positive nuclei, the activation of trophoblast genes and accelerated the exit from naive pluripotency during trophoblast fate induction. Single-cell RNA-seq analyses showed that an increased proportion of PRC2i-treated cells aligned with the human embryo trophectoderm and trophoblast lineage compared to control, in line with increased trophoblast induction upon PRC2i. To further investigate a role for PRC2 in trophoblast specification and morphogenesis, we used human blastoids as a 3D assay. PRC2i increased the proportion of TROP2 and GATA3-positive trophoblast-like cells during blastoid formation, and decreased the ratio of epiblast-like cells. Additionally, we measured blastocyst-like cavity formation in blastoids, whose expansion is critical for embryonic uterine implantation, and observed that blastoid cavities appeared earlier upon PRC2i, supporting an acceleration of trophoblast specification and epithelial morphogenesis. Thus, our results show that naive hPSCs are not epigenetically unrestricted, and instead possess PRC2 as a chromatin barrier to the trophoblast fate and to the formation of functional trophoblast with trophectoderm-like morphogenetic functions.

Funding Source: This work is supported by the FWO, KU Leuven, BBSRC, MRC and the Wellcome Trust.
Keywords: Trophoblast, Human blastoids, Polycomb Repressive Complex 2

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METTL3-MEDIATED RNA METHYLATION ORCHESTRATES TRANSCRIPTIONAL DORMANCY DURING PAUSED PLURIPOTENCY

Collignon, Evelyne A. - *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*
Cho, Brandon - *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*
Fothergill-Robinson, Julie - *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*
Furlan, Giacomo - *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*
Ross, Robert - *N/A, Thermo Fisher Scientific, Franklin, MA, USA*
Limbach, Patrick - *Department of Chemistry, University of Cincinnati, OH, USA*
Ramalho-Santos, Miguel - *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada*

Abstract: Development is often assumed to be a sequential unfolding of genetic programs towards increased complexity and occurring with a very stereotypical timing. However, embryos across all lineages of metazoans can enter reversible states of developmental pausing in response to adverse environmental conditions. In mammals, pausing manifests as a delayed implantation of the blastocyst, the source of embryonic stem cells (ESCs). Paused pluripotency can be induced in mouse blastocysts and ESCs by inhibition of the mTOR pathway and is characterized by a marked global decrease in biosynthetic activity, including gene transcription. The molecular mechanisms by which this dormant cellular state is achieved remain largely unknown. Here we show that RNA methylation by Mettl3 is essential for transcriptional dormancy and maintenance of the paused pluripotent state in

vitro and in vivo. Mass spectrometry and RNA immunoprecipitation-sequencing revealed an increase in m6A RNA methylation in paused ESCs. We found that knockout of the RNA methyltransferase Mettl3 suppresses pausing of ESC proliferation and leads to premature death of paused blastocysts. Analyses of nascent and steady-state RNA levels revealed a de-repression of transcription in paused Mettl3-/- cells. Integration of several datasets in vitro and in vivo identified the transcriptional amplifier and oncogene Mycn as a key "anti-pausing factor" regulated by m6A mRNA methylation. We found that Mett3-mediated methylation at a specific site of the Mycn mRNA is essential for its destabilization, which in turns mediates suppression of nascent transcription and proliferation in paused conditions. Our results highlight an intricate feedback between signaling, regulation of RNA stability and nascent transcription during pausing, with Mettl3 as an essential integrator and Mycn as a key downstream anti-pausing factor. Our most recent data will be discussed. These findings shed light on the mechanisms that underlie tuning of global transcriptional output during mammalian developmental pausing, and that may be redeployed in adult stem cells or during cancer dormancy.

Funding Source: This work was supported by a fellowship from the Belgian American Educational Foundation and project grants from the Canadian Institutes for Health Research (420231 and 178094).
Keywords: RNA methylation, Paused pluripotency, Transcription regulation

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LIPIDS MAINTAIN GENOMIC STABILITY AND FULL POTENCY OF MURINE EMBRYONIC STEM CELLS

Zhong, Liangwen - *Reproductive Medicine, Weill Cornell Medical College, New York, NY, USA*
Gordillo, Miriam - *Department of Surgery, Weill Cornell Medicine, New York, NY, USA*
Wang, Xingyi - *Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory, New York, NY, USA*
Qin, Yiren - *Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA*
Huang, Yuanyuan - *Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA*
Soshnev, Alexey - *Laboratory of Chromatin Biology & Epigenetics, The Rockefeller University, New York, NY, USA*
Kumar, Ritu - *Gladstone Institutes, Gladstone Institutes, San Francisco, CA, USA*
Nanjangud, Gouri - *Molecular Cytogenetics Core, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
James, Daylon - *Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA*
Allis, C. David - *Laboratory of Chromatin Biology & Epigenetics, The Rockefeller University, New York, NY, USA*
Zhang, Chao - *Department of Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA*
Evans, Todd - *Department of Surgery, Weill Cornell Medicine, New York, NY, USA*
Carey, Bryce - *Laboratory of Chromatin Biology & Epigenetics, The Rockefeller University, New York, NY, USA*
Wen, Duancheng - *Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY, USA*

Abstract: While Mek1/2 and Gsk3β inhibition ("2i") supports the maintenance of mouse ES cells (ESCs) in a homogenous naïve state, prolonged culture in 2i results in aneuploidy and DNA hypomethylation that impairs developmental potential. Additionally, 2i fails to support derivation of fully potent female ESCs. Lipids play vital roles in cellular homeostasis yet are typically considered passive in cell-fate determination. Here we find that ESCs cultured in 2i/LIF supplemented with lipid-rich albumin (AlbuMAX) undergo pluripotency transition yet maintain genomic stability and developmental (4n-competency) over long-term culture. Mechanistically, lipids in AlbuMAX impact intracellular metabolism including nucleotide biosynthesis, lipid biogenesis, and TCA cycle intermediates. AlbuMAX induces pluripotency transition which can be restrained through manipulating Fgf/Erk signaling. Importantly, both male and female "all-ESC" mice can be generated from de novo derived ESCs using AlbuMAX-based media. These findings underscore the importance of lipids to pluripotency and link nutrient cues to genome stability in early development.

Keywords: Mouse embryonic stem cells, Lipids, Pluripotency transition, Genomic stability, Erk1/2 signaling, 2i medium, X chromosome loss, Female All-ESC mice, Developmental potency, Naïve pluripotency, Formative pluripotency, Nucleotide pool, Telomere shortening, Zscan4

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H3K36 METHYLATION MAINTAINS CELL IDENTITY BY REINFORCING CELL TYPE-SPECIFIC ENHANCERS AND OPPOSING ALTERNATIVE LINEAGE ENHANCERS

Hoetker, Michael S. - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Yagi, Masaki - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Di Stefano, Bruno - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Cristea, Simona - *Department of Data Sciences, Dana-Farber Cancer Institute, Boston, MA, USA*
Wong, Lai Ping - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Langerman, Justin - *Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA, USA*
Huebner, Aaron - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Charlton, Jocelyn - *Department of Stem Cell and Regenerative Biology, Harvard University, Boston, MA, USA*
Sadreyev, Ruslan - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*
Meissner, Alexander - *Department of Genome Regulation, Max Planck Institute for Molecular Genetics, Berlin, Germany*
Michor, Franziska - *Department of Data Sciences, Dana-Farber Cancer Institute, Boston, MA, USA*
Plath, Kathrin - *Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA, USA*
Hochedlinger, Konrad - *Department of Molecular Biology, Harvard Medical School, Boston, MA, USA*

Abstract: While development and differentiation are well-characterized epigenetic processes, the mechanisms that subsequently maintain specialized cell states remain largely unexplored. Here, we employed histone mutants to uncover a crucial role for H3K36 methylation in the maintenance of cell identities across diverse developmental contexts. Focusing on the experimentally induced conversion of fibroblasts to pluripotent stem cells, we

showed that H3K36M-mediatd disruption of H3K36 methylation endowed intermediate populations with a plastic epithelial state poised to acquire pluripotency in virtually every cell. Mechanistically, H3K36M led to the downregulation of mesenchymal and TGFb-associated genes by depleting H3K36me2 and H3K27ac at somatic enhancers, resulting in their decommissioning. In parallel, H3K36M led to the induction of epithelial and stem cell genes by facilitating an accessible and hypomethylated chromatin state permissive for pluripotency factor binding. Together, our findings reveal a previously unappreciated, dual role for H3K36 methylation in the maintenance of cell identity, by integrating a key developmental pathway into sustained activity of cell type-specific enhancers, and by opposing the activation of alternative lineage enhancers. Our results are highly relevant for understanding the impact of altered H3K36 methylation patterns on physiological and pathological cell fate transitions, including development, tissue regeneration and cancer.

Keywords: Cell Identity, Epigenetic regulation, Enhancer biology

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EXOGENOUS OCT4/POU5F1 IS NOT REQUIRED TO PRODUCE INDUCED EXTRAEMBRYONIC ENDODERM STEM CELLS DURING SOMATIC CELL REPROGRAMMING

Moauro, Alexandra - *Physiology, Michigan State Univeristy, Lansing, MI, USA*
Halbisen, Mike - *Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA*
Ralston, Amy - *Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA*

Abstract: Retroviral reprogramming of mouse fibroblasts leads to formation of two distinct stem cell types: induced pluripotent stem (iPS) cells and induced extraembryonic endoderm stem (iXEN) cells. Both iPS and iXEN cells can proliferate and differentiate in a lineage-appropriate manner, indicating that they are authentic stem cell lines. Recently, it was shown that exogenous Sox2, Klf4 and c-Myc (SKM) are sufficient to induce the formation of iPS cells when delivered using lentivirus instead of MMLV-derived retrovirus overexpression systems. However, the formation of iXEN cells in this context was not examined. We therefore sought to test the hypothesis that SKM can induce formation of iXEN cells, in the absence of exogenous Oct4. In support of this hypothesis, we discovered that SKM-lentiviral reprogramming produces iXEN as well as iPS cells. SKM-induced iXEN cell lines express the same markers and exhibit similar differentiation potential as OSKM-induced iXEN and embryo-derived XEN cell lines. This is significant because SKM has been proposed to engage the pluripotency pathway more specifically than OSKM. We further discovered that endogenous Oct4 is induced by either SKM or OSKM. Since Oct4 is expressed in, and required for, both pluripotent and XEN populations in the embryo, we hypothesized that reprogramming leads to expression of Oct4 in iPS and iXEN cells. Using flow cytometric cell sorting, we sorted single cells expressing endogenous Oct4-eGFP, and then evaluated the morphologies, gene expression, and developmental potential of these clonal cell lines. We found that, indeed, a subset of OCT4-eGFP-positive cells are fated for iXEN formation. This indicates that endogenous Oct4 is expressed in cells fated for iXEN and that OCT4 helps specify multiple cell types in reprogramming, as it does in the embryo.

Funding Source: Funding for this work was provided by NIH R35GM131759 awarded to A.R.
Keywords: Reprogramming, OCT4, iXEN

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ENHANCED MATURATION OF HUMAN STEM CELL DERIVED INTERNEURONS BY MTOR ACTIVATION

Manley, William - *Child and Adolescent Psychiatry, The Children's Hospital of Philadelphia, PA, USA*
Anderson, Stewart - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Chu, Jianhua - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Fitzgerald, Megan - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Fitzgerald, Shane - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Goldberg, Ethan - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Johnson, Brian - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*
Naung, Harrison - *Child and Adolescent Psychiatry, Children's Hospital of Philadelphia, PA, USA*

Abstract: The use of stem cell derived neurons for cell-based therapies is limited by their protracted maturation. We present a novel approach for accelerating the post-mitotic maturation of human stem cell derived interneurons via the constitutive or transient activation of mTOR signaling. For constitutive activation, Lox sites were placed within PTEN, a key mTOR inhibitor, in a cortical interneuron (CIn) reporter line in which GFP is expressed by the Lhx6 locus. Following directed differentiation and purification by FACS, the CIns were exposed to Cre-expressing lentivirus, then transplanted into mouse neocortex or plated onto cultured rat neocortex. Input synaptogenesis and dendritogenesis was greatly enhanced in the PTEN-deleted CIns. Whole-cell recording of the PTEN-deleted CIns in slices of transplanted neocortex revealed multiple indices of enhanced maturation. Finally, we observed similar effects using transient, doxycycline-inducible activation of myristoylayed AKT. Ongoing experiments are evaluating neuronal maturation by single nuclei RNA-seq (sNuc-Seq) and pseudotime analysis of neurons dissociated from medial ganglionic eminence like IPSC-derived spheroids with doxycycline-inducible activation of AKT. We thus present an inducible, reversible approach for accelerating the maturation of human stem cell derived CIns, which can be used to study the influences of this disease-related signaling system in human neurons.

Keywords: maturation, stem, interneurons

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DICHOTOMOUS ROLE OF SHP2 FOR NAIVE AND PRIMED PLURIPOTENCY MAINTENANCE IN EMBRYONIC STEM CELLS

Kim, Seong-Min - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kwon, Eun-Ji - *College of Pharmacy, Seoul National University, Seoul, Korea*

Kim, Yun-Jeong - *College of Pharmacy, Seoul National University, Seoul, Korea*
Go, Young-Hyun - *Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea*
Oh, Ji-Young - *College of Pharmacy, Seoul National University, Seoul, Korea*
Park, Seokwoo - *Department of Biomedical Sciences, Seoul National University, Seoul, Korea*
Do, Jeong-Tae - *Department of Stem Cell and Regenerative Biology, Konkuk University, Seoul, Korea*
Kim, Keun-Tae - *College of Pharmacy, Seoul National University, Seoul, Korea*
Cha, Hyuk-Jin - *College of Pharmacy, Seoul National University, Seoul, Korea*

Abstract: The requirement of the Mek1 inhibitor (iMek1) during naïve pluripotency maintenance results from the activation of the Mek-Erk1/2 (Mek/Erk) signaling pathway upon leukemia inhibitory factor (LIF) stimulation. Through a meta-analysis of previous genome-wide screening for negative regulators of naïve pluripotency, Ptpn11 (encoding the Shp2 protein, which serves both as a tyrosine phosphatase and putative adapter), was predicted as one of the key factors for the negative modulation of naïve pluripotency through LIF-dependent Jak/Stat3 signaling. Using an isogenic pair of naïve and primed mouse embryonic stem cells (mESCs), we demonstrated the different role of Shp2 in naïve and primed pluripotency. Loss of Shp2 increased naive pluripotency by promoting Jak/Stat3 signaling and disturbed in vivo differentiation potential. In sharp contrast, Shp2 depletion significantly impeded the self-renewal of ESCs under primed culture conditions, which was concurrent with a reduction in Mek/Erk signaling. Similarly, upon treatment with an allosteric Shp2 inhibitor (iShp2), the cells sustained Stat3 phosphorylation and decoupled Mek/Erk signaling, thus replacing the use of iMek1 not only for maintenance but also for the establishment of naïve ESCs through reprogramming. Taken together, our findings highlight the differential roles of Shp2 in naïve and primed pluripotency and proposes the usage of iShp2 instead of iMek1 for the efficient maintenance and establishment of naïve pluripotency.

Keywords: Shp2 activity balances Naïve and Primed pluripotency by regulating MAPK and Jak/Stat3 pathway in mESCs, Naïve pluripotency, Primed pluripotency

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AN EXPANDED INTERACTOME OF THE SILENCING FACTOR SPEN IDENTIFIES NOVEL FACTORS THAT POTENTIALLY ACT IN X CHROMOSOME INACTIVATION

Robert-Finestra, Teresa - *Developmental Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Tan, Beatrice F. - *Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Mira-Bontenbal, Hegias - *Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Gontan, Cristina - *Department of Developmental Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Gribnau, Joost - *Developmental Biology, Erasmus University Medical Center, Rotterdam, Netherlands*

Abstract: To ensure X-linked gene dosage compensation between female (XX) and male (XY) mammalian cells, one X chromosome undergoes X chromosome inactivation (XCI) in female cells. This process is a paradigm of epigenetic control of gene regulation. XCI is tightly regulated throughout development by many different factors including the long non-coding RNA, Xist, and its anti-sense transcript, Tsix. At the onset of XCI, Xist is upregulated from the future inactive X (Xi) chromosome, overcoming Tsix repression, and establishes several layers of repressive epigenetic modifications. Different studies aimed at identifying primary X silencing factors uncovered a role for SPEN (also known as SHARP) as a direct Xist interacting protein required for X-linked gene silencing and establishment of the Xi, mostly via recruitment of repressor proteins SMRT and HDAC3, leading to histone deacetylation and RNA PolII exclusion. Recently we showed that SPEN is not only crucial for X-linked gene silencing, but is also essential for Xist upregulation, predominantly via Tsix silencing but also via Xist RNA stabilization. While SPEN removal completely abolishes X-linked gene silencing, HDAC3 deletion causes delayed silencing. These observations indicate that SPEN might recruit still unknown HDAC3-independent silencing factors. Therefore, we performed full length SPEN immunoprecipitation followed by mass-spectrometry and compared the resulting expanded SPEN-interactome in differentiating female mouse embryonic stem cells (ESCs) undergoing XCI with XCI-deficient XO ESCs. This analysis reveals several XCI-specific SPEN interactors that might play a role in X-linked silencing. Moreover, our data indicates a role for SPEN in Xist RNA splicing. This study aims to further clarify the chronology of events that render the X chromosome epigenetically inactive.

Keywords: Xist, long non-coding RNAs, SPEN/SHARP, Embryonic stem cells

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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NARROWING A CONSENSUS DEFINITION FOR ENDOTHELIAL PROGENITOR CELLS

Styke, Cassandra - *Faculty of Medicine, The University of Queensland Diamantina Institute, Woolloongabba, Australia*
Kaur, Simranpreet - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*
Zhao, Jilai - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*
Sim, Seen Ling - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*
Shafiee, Abbas - *Queensland University of Technology, Brisbane, Australia*
Zhou, Chenhao - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*
Wong, Ho Yi - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*
Harvey, Richard - *Victor Chang Cardiac Research Institute and St. Vincent's Medical School and School of Biotechnology and Biomolecular Science, UNSW, Sydney, Australia*
Khosrotehrani, Kiarash - *Faculty of Medicine, The University of Queensland Diamantina Institute, Brisbane, Australia*

Abstract: Endothelial stem/progenitor cells (EPCs) have long been investigated as targets for a variety of clinical applications, however, lack of a uniform definition hinders their use. Several genes and surface markers have been used to define EPCs, leading to various putative EPC populations. We hypothesized that overlapping previously used EPC markers would allow a consensus definition to be validated by functional analysis. Here we examined Procr, PDGFRα, CD157, Abcg2 (a side population marker), Sox18,

and EVPs (VE Cadherin+Hematopoietic Lineage-CD34+CD31lo) as potential EPC markers in the murine aorta. Flow cytometry analysis showed that Procr and PDGFRα were consistently more likely to be co-expressed with EVPs than mature differentiated endothelial (D) cells (VECad+Lin-CD34+CD31+) in the aorta of adult C57Bl/6 mice (78.04% and 82.28% compared to 54.22% and 24.65% of cells, respectively; n=5; $p < 0.001$). Single-cell RNA-sequencing confirmed clustering of Procr and PDGFRα with EVPs, while CD157, Sox18 and Abcg2 clustered with D cells. Immunofluorescence staining showed that Procr+ EVPs co-localized with YFP+ endothelium in the aortae of Cdh5-CreERT2/Rosa-EYFP reporter mice. Aortic Procr+ EVPs had greater endothelial colony forming capability in vitro (2.74% of wells) than either Procr- EVPs (0.69%) or Procr+/- D cells (0%; n = 3) when cultured in Matrigel, as well as greater engraftment and tube-formation ability when implanted in a collagen matrix in vivo. Lineage tracing via flow cytometry of 4-week-old developing PDFGRα-MerCreMer/Rosa-EYFP mice showed that aortic YFP+ EVPs at day 0 (D0) differentiated into D cells by D84 (0.74% D cells at D0, 4.67% at D84; n = 5; $p < 0.001$). Further lineage tracing confirmed that YFP+ EVPs at D0 from full skin excisional wounds of adult PDFGRα-MerCreMer/Rosa-EYFP mice differentiated into D cells by D5 (0.04% D cells at D0, 1.19% at D5; n = 7; $p = 0.0015$), suggesting that mesenchymal marker PDGFRα marks an EPC population capable of endothelial fate in both homeostasis and injury. The characteristics displayed by Procr+ and PDGFRα+ EVPs suggest these may mark a true EPC population. A consensus definition would allow therapeutic strategies to specifically target EPCs in a variety of clinical applications and allow insights into vascularization in tissue- and bio-engineering fields.

Keywords: Endothelial progenitor cells, Vascularization, Angiogenesis

TOPIC: GERMLINE

806

EFFECTS OF AGING ON THE SPERMATOGONIAL STEM CELL SYSTEM

Konishi, Natsuki - *Graduate School of Science and Technology, Shinshu University, Ueda, Japan*
Kakuta, Shigeru - *Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Japan*
Kyuwa, Shigeru - *Laboratory of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Japan*
Takashima, Seiji - *Institute for Biomedical Science, Interdisciplinary Cluster for Cutting Edge Research, Shinshu University, Ueda, Japan*

Abstract: Spermatogenesis is sustained by the self-renewal and differentiation of spermatogonial stem cells (SSCs). SSCs self-renewal is mediated by Glial cell line-derived neurotrophic factor (GDNF), while differentiation depends on retinoic acid (RA). Following differentiation, SSC clones undergo meiosis cyclically and develop a spatiotemporal association of germ cell known as the “seminiferous epithelium cycle”. We previously identified fibroblast growth factor (FGF) 2 as another bona fide self-renewal factor for SSCs and revealed the functional differences between these factors. In particular, GDNF-dependent spermatogonia (G-SPG) exhibit higher stem cell activity and rapid proliferation, while FGF2-dependent spermatogonia (F-SPG) exhibit lower stem cell activity and differentiation-prone phenotype with higher expression of retinoic acid receptor gamma (RARG), a receptor for RA. However, the role of these subpopulations in vivo remains to be undetermined. To understand the behavior of two subpopulations in vivo, we analyzed the SSC dynamics and gene expression in postnatal mouse testes at various developmental stages. We found the expansion of GFRA1+RARG- spermatogonia (corresponding to G-SPG) in neonatal testis compared to pubertal testis. this was consistent with the dominant expression of Gdnf compared to Fgf2 in neonatal testis. By contrast, aged testis exhibits the expansion of GFRA1+RARG+ spermatogonia (corresponding to F-SPG) and the dominant expression of Fgf2 compared to Gdnf. Additionally, we also found that the wavelength of seminiferous epithelial cycle was significantly shortened in aged testis. These data suggest that aging affects not only relative population of SSC subpopulations but also the differentiation dynamics of these subpopulations. In the presentation, we also focused on factors associated with the senescence-associated secretory phenotype (SASP) and examined their effects on SSC dynamics.

Keywords: spermatogonial stem cell, self-renewal factor, aging

TOPIC: NEURAL

808

COMPARING THE TRANSCRIPTOMIC TRAJECTORIES OF DIFFERENTIATING IPSC-SPINAL MOTOR NEURONS WITH THAT OF DEVELOPING HUMAN SPINAL CORD VENTRAL HORN

Chun, Changho - *Bioengineering, University of Washington, Belleuve, WA, USA*
Bothwell, Mark - *Physiology and Biophysics, University of Washington, Seattle, WA, USA*
Mack, David - *Rehabilitation Medicine, University of Washington, Seattle, WA, USA*
Regier, Mary - *Bioengineering, University of Washington, Seattle, WA, USA*
Smith, Alec - *Physiology and Biophysics, University of Washington, Seattle, WA, USA*

Abstract: The progressive pathologies of several neuromuscular diseases like amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth (CMT) are driven by defects in more than one neuronal subtype. The interaction of these neuronal subpopulations in the ventral horn of the spinal cord is essential for normal development, long-term survival, and function throughout life. Our group has developed protocols to differentiate human iPSCs into motor, sensory, and interneurons in culture to investigate the molecular drivers of ALS and CMT, but the ratios of cell types and their transcriptomic profiles do not mirror embryonic development. In this study, we identified a longitudinal transcriptomic signature of differentiating ventral spinal cord motor neurons and compared it with the profile of the native fetal spinal cord at different gestational stages. Preliminary analyses found that at day 32 of differentiation, the culture contains 37.7% of spinal motor neurons having a mostly cervical and brachial regional identity. Thoracic v3 interneurons and hindbrain sensory neurons made up 9.3% and 6.2% of culture, respectively. The UMAP distribution showed clear segregation of the non-neuronal cells from neuron populations, which was mostly astrocyte progenitors expressing FABP7, C1orf61, Vimentin but not MAP2. Additional experiments underway include the analyses of the single-cell transcriptomes of developing iPSC-motor neurons at days 0, 6, 12, 18, 21, 24,

27, 30, 35, 40, 45 to map their trajectories for the side-by-side comparison with the data obtained from the human fetal spinal cord ventral horn. With the comprehensive transcriptomic information, we will establish an iterative workflow of identifying fetal neuronal clusters with distinct transcriptional signatures that then drives the refinement of our in vitro differentiation protocol(s). The overarching goal is to investigate the differences in transcriptome profiles between healthy spinal neuron populations and those bearing mutations known to induce neuromuscular disease, to identify the gene expression changes driving disease onset and progression.

Keywords: Developmental trajectory of iPSC-neurons, Fetal spinal cord vs. iPSC-spinal neurons, Neuronal diversity of human spinal cord

810

A 3D-PRINTED DEVICE PROVIDING MECHANICAL STRAIN FOR MATRIGEL-INCORPORATED ORGANOIDS

Abou-Sharieha, Samah - *Department of Material Science and Technology, Uppsala University, Uppsala, Sweden*
Atif, Abdul-Raouf - *Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden*
Mestres, Gemma - *Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden*
Tenje, Maria - *Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden*

Abstract: Human pluripotent stem cell (hPSC)-derived cortical organoids mimic the diversity of mature cell types and the cytoarchitecture that oligodendrocytes encounter in vivo. Still, these organoids lag behind in yielding reproducible mature oligodendrocytes, the myelinating glia of the central nervous system (CNS). Static tensile strains within the range observed in vivo (10-15%) were found to significantly decrease proliferation and increase differentiation of oligodendrocyte-progenitor cells (OPCs) into myelinating oligodendrocytes in a study of which model was OPCs grown in 2-dimensional culture. So far, no work has been done to provide mechanical strain for 3-dimensional cortical organoids so that the strain is transferred to cell nucleus where it modifies gene expression in a way consistent with enhanced oligodendrocyte differentiation. We 3D-printed a device made of polylactic acid (PLA), a biocompatible material, in which magnets coated with paralyene were incorporated. We loaded 20 µm in diameter fluorescent beads in Matrigel and adjusted the force by which the magnets hit the Matrigel so that 10-15% strain was achieved. This platform enables studies focused on the role of mechanical strain in the differentiation of cortical organoids.

Keywords: organoids, 3D-printed device, mechanical strain

TOPIC: PLURIPOTENT STEM CELLS

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SYSTEMATIC DISSECTION OF AN EXTENDED NAÏVE PLURIPOTENCY GENE REGULATORY NETWORK

Cerron Alvan, Luis Miguel - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Lab, Vienna, Austria*
Leeb, Martin - *Microbiology, Immunobiology & Genetics, Max Perutz Labs, Vienna, Austria*

Abstract: How do pluripotent stem cells define their identity to go further into proper development? To answer this question the exit from naïve pluripotency model has been extensively studied, nevertheless the involving gene regulatory networks (GRNs) still remain not fully elucidated. With the purpose of studying an extended naïve GRN, a set of 496 naïve pluripotency associated genes, hereafter named NAGs, have been identified as tightly associated to known core pluripotency markers in mouse. This gene set involves not only transcription factors but also epigenetic factors and genes not previously reported as naïve pluripotency-related. Moreover, the NAGs follow a similar expression dynamic in vitro and in vivo during epiblast transition, suggesting in vivo relevance. Additionally, these genes exhibit similar regulation in human and macaque, suggesting potential relevance across mammalian species. These aspects make the NAGs an interesting gene set to be studied. To identify which genes within the NAGs have an actual and fundamental role in the naïve state, we proposed to perform both CRISPR activation and interference-based screens. For this purpose, dCas9 fused to transcriptional effectors and a customized pooled gRNA library were delivered into the cell line reporter "Rex1-GFP". This cell line loses GFP signal under cell differentiation, enabling the monitoring of the exit from naïve pluripotency in high resolution. Furthermore, in order to explore interactions with pluripotency-related pathways, the setup of these screens also included the individual addition of components known to maintain the naïve pluripotent state such as LIF, PD0325901 and CHIR99021. Thus, we expect to identify novel gene roles that can contribute to a better understanding of the underlying mechanisms involved in the naïve pluripotent state.

Keywords: exit from naïve pluripotency, naïve associated genes, CRISPRa CRISPRi

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KLF5 REWIRES NANOG TO ACTIVATE HUMAN NAÏVE SPECIFIC ENDOGENOUS RETROVIRUS LTR7YS AND ENFORCE THE NAÏVE PLURIPOTENCY

Liang, Hongqing - *School of Medicine, Zhejiang University, Hangzhou, China*

Abstract: The endogenous retroviruses (ERVs) have been reported to play a role in the pre-implantation development of mammalian embryos. In early human embryogenesis, different ERV sub-families are activated in a highly stage-specific manner. How the specificity of ERV activation is achieved remains largely unknown. Here, we demonstrate the mechanism of how LTR7Y, the human morula-blastocyst-specific human endogenous retrovirus subfamily H (HERVH) long terminal repeat (LTR), are activated by naïve pluripotency transcription network. We find that KLF5 interacts with and rewires NANOG to bind and regulate the LTR7Y sub-family; in contrast, the primed-specific LTR of HERVH, LTR7s are preferentially bound by NANOG in the absence of KLF5. The specific activation of chromatin-based function of LTR7Ys by KLF5 and NANOG in pluripotent cells led to the up-regulation of naïve and trophectoderm-related genes, participating in the human-specific naïve pluripotency regulation and promoted cell potential towards trophectoderm. Our study reveals that ERVs are activated by the cell state-specific transcription machinery, and

functionally contribute to stage-specific transcription network and cell potency.

Keywords: endogenous retroviruses, transcription rewiring, naïve ESC and trophectoderm potency

POSTER SESSION II: EVEN

4:00 PM – 5:00 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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TRACKING THE REAL-TIME PROGRESS OF HPSC-DERIVED CELL THERAPIES IN HPSCREG

Mah, Nancy - *Biomedical Data and Bioethics Group, Fraunhofer-Institute for Biomedical Engineering, Sulzbach, Germany*
Kobold, Sabine - *Biomedical Data and Bioethics Group, Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany*
Bultjer, Nils - *Biomedical Data and Bioethics Group, Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany*
Mueller, Sabine - *Biomedical Data and Bioethics Group, Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany*
Stacey, Glyn - *International Stem Cell Banking Initiative, Barley, UK*
Kurtz, Andreas - *Biomedical Data and Bioethics Group, Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany*

Abstract: The human pluripotent stem cell registry (hPSCreg®; https://hpscreg.eu) was founded in 2007 with the support of the European Commission to uphold the ethical provenance of human embryonic stem cell research in the European Union. The Registry has since evolved into a leading world-wide resource for human pluripotent stem cell (hPSC) lines, including human induced pluripotent stem cell (hiPSC) lines. It has detailed information on quality-controlled stocks of hundreds of hiPSC lines from major European projects such as EBiSC, HipSci and StemBANCC. As the application of hPSC lines moves towards clinical treatments, hPSCreg® aims to expand the existing resource to document the properties of hPSC lines intended for clinical translation in a "regulatory-primed" hPSCreg cell line registry. Finally, the progressive application of these lines in cell-based medicines can be monitored through the use of hPSC-derived products in clinical studies. To this end, hPSCreg® has established and proactively maintains a manually curated clinical studies database for hPSC-based therapies (https://hpscreg.eu/browse/trials) that is updated on an ongoing basis. The database only includes clinical studies that have been registered at national clinical trial registries and involve the use of hPSC-derived cells for interventional treatment. As of March 2022, the clinical study database holds 96 clinical studies spanning 11 clinical indications grouped by different ICD-10 chapters and associated disease areas. These results indicate a steep rise since hPSCreg® started tracking clinical trials in 2018. In the present work, we provide an update of the database content and plans for its continued development for the stem cell community and public-at-large.

Funding Source: European Commission Horizon2020 Project ID: 726320
Keywords: clinical trials, stem cell-based treatments, knowledgebase

TOPIC: HEMATOPOIETIC SYSTEM

208

NON-GENOTOXIC RESTORATION OF THE HEMATOLYMPHOID SYSTEM IN FANCONI ANEMIA

Czechowicz, Agnieszka D. - *Pediatrics, Stanford University, Palo Alto, CA, USA*
Chan, Yan Yi - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Nofal, Rofida - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Ho, Pui Yan - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Thongthip, Supawat - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Rayburn, Maire - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Swartzrock, Leah - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Scheck, Amelia - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*
Weinberg, Kenneth - *Pediatrics - Ped Stem Cell Transplantation, Stanford University, Stanford, CA, USA*

Abstract: Fanconi Anemia (FA) is a grievous genetic disease predisposing patients to bone marrow failure and neoplasia. The hematolymphoid manifestations of the disease can be cured by allogeneic hematopoietic stem cell transplantation (HSCT), however FA patients have extremely high rates of malignancies post HSCT due to their underlying defects in DNA damage repair that make them exceptionally sensitive to current genotoxic chemotherapy and/or irradiation-based HSCT conditioning regimens with high malignancy rates post HSCT. Hence, we have directed our efforts to establish alternative non-genotoxic HSCT strategies for FA. Our group has previously developed several monoclonal antibody (mAb) based conditioning strategies for non-genotoxic HSCT by targeting the HSC-cell surface receptor CD117 pre-clinically in various disease models. To translate this approach to FA, we evaluated the efficacy and toxicity of different αCD117 mAb strategies and immunosuppression for HSCs depletion and explored strategies to establish therapeutic donor hematopoiesis post HSCT in FA mice. Interestingly, Interestingly, we found that HSC depletion and donor chimerism with disease correction were achieved only when αCD117 mAbs were augmented with complementary agents of CD47 mAb blockade or antibody-drug-conjugation (ADC).. Surprisingly, animals conditioned with immunosuppression alone showed similar donor chimerism to those conditioned with αCD117 mAbs + immunosuppression, with increasing donor chimerism to >80% suggesting that immunosuppression-only conditioning may be sufficient in FA transplant settings. Our novel findings suggest that if sufficient immunosuppression is given to obtain initial donor HSC engraftment, resulting turnover of a majority of the hematolymphoid system can occur likely due to the survival advantage of WT HSCs over FA HSCs. Such a non-toxic

all antibody-based conditioning strategy could be transformative for FA patients. These promising findings encourage investigating parallel treatment strategies in patients which will be explored in a recently opened clinical trial (NCT03814408).

Funding Source: Fanconi Anemia Research Fund (SPO 133140) ; Rocket Pharmaceuticals Inc. (SPO 138540)
Keywords: Fanconi Anemia, Hematopoietic Stem Cell Transplantation, Non-Genotoxic Conditioning

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MODELING OF TYROSINE-KINASE INHIBITOR-RESISTANT CHRONIC MYELOID LEUKEMIA DERIVED INDUCED PLURIPOTENT STEM CELLS

Prem Kumar, BenjaminSathya Bama, Esther - *Haematology, Christian Medical College, Vellore, India*
Prem Kumar, Benjamin - *Hematology, Christian Medical College, Vellore, India*
Joshi, Gaurav - *Hematology, Christian Medical College, Vellore, India*
Rajamani, Bharathi - *Hematology, Christian Medical College, Vellore, India*
Nandi, Krittika - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Rani, Sonam - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Velayudhan, Shaji - *Hematology, Christian Medical College, Vellore, India*
Balasubramanian, Poonkuzhali - *Hematology, Christian Medical College, Vellore, India*

Abstract: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the BCR-ABL1 fusion gene with constitutively active tyrosine kinase activity. Although the tyrosine kinase inhibitors (TKI) have revolutionized the treatment for CML, withdrawal of TKI therapy in patients on deep molecular response causes disease relapse, primarily due to the persistence of leukemic stem cells (LSCs) that are insensitive to TKI. As LSCs are a rare population of cells, it is challenging to understand the molecular basis of the disease relapse to tailor strategies to eliminate them selectively. Patient-derived induced pluripotent stem cells are increasingly used for disease modeling and high through drug screening. We reprogrammed cryopreserved CD34+ from CML patient with good response to imatinib (IM) (n=2), and a non-responder (n=1). The CD34+ cells were expanded in SFEM II supplemented with CD34+ Expansion Supplement (10X) including UM729 for 3-days followed by nucleofection with episomal reprogramming plasmids as described previously (Manian et al., 2018). After nucleofection, CML CD34+ were reprogrammed with or without IM (10μM). We recently showed that the addition of IM improved the efficiency and maintenance of CML iPSCs (Benjamin et al. AACR annual meeting abstracts, 2022). Here, we reprogrammed the CML CD34+ in the presence and absence of IM. To our surprise, the CML iPSC colonies formed earlier (21 days vs. 38 days) in the CD34+ cells derived from the IM non-responder reprogrammed in the presence of IM. No iPSC colonies formed from the imatinib responder-derived CD34+ cells. Alkaline phosphatase staining revealed that the addition of IM during reprogramming of refractory CD34+ cells enhanced the iPSC colony formation. These results suggest that CML CD34+ from IM refractory CML patients are resistant to TKI and survive in the presence of IM. This proof-of-concept study identified a unique CML LSCs mimetic model that could serve as an excellent platform for screening small molecules to eliminate CML LSCs selectively.

Funding Source: Centre of Excellence grant from Department of Biotechnology India: BT/COE/34/SP13432/2015 and Indian Council of Medical Research Centre for Advanced Research grant 70/14/14-CAR to Dr. Poonkuzhali Balasubramanian
Keywords: Chronic myeloid leukemia, Leukemic stem cells, Induced pluripotent stem cells

TOPIC: MUSCULOSKELETAL

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MIR144 OVEREXPRESSING MESENCHYMAL STEM CELL DERIVED SECRETOME PROTECT OGCS FROM CYCLOPHOSPHAMIDE INDUCED CYTOTOXICITY BY AUGMENTING CELL PROLIFERATION AND INHIBITING APOPTOSIS

Liakath Ali, Farzana - *Obstetrics and Gynecology, University of Chicago, IL, USA*
Al-Hendy, Ayman - *Obstetrics and Gynecology, University of Chicago, IL, USA*
Mousaei, Mohammad - *Obstetrics and Gynecology, University of Chicago, IL, USA*
Park, Hang Soo - *Obstetrics and Gynecology, University of Chicago, IL, USA*
Seok, Jin - *Obstetrics and Gynecology, University of Chicago, IL, USA*

Abstract: The clinical manifestation of Premature Ovarian Insufficiency (POI) is largely attributed due to the follicular atresia caused by excessive senescence, apoptosis, and loss of ovarian granulosa cells (OGC) resulting in decreased number of follicles. microRNA 144 has been reported to be downregulated in the plasma of POI patients and previous studies have reported that miRNA 144-5p can be derived from BM-MSC derived exosomes. This study aims to enhance the therapeutic effect of genetically modified BM-MSC using an in vitro POI model. The human BM-MSC were successfully transfected using lentivirus construct carrying miR144-5p with Green fluorescent protein (GFP) tag with scramble control. The transfected clones were confirmed by fluorescent microscopy and sorted using magnetic assisted cell sorting. The purified clones over expressing miR144-5p were confirmed by immunophenotyping and quantitative PCR. The transfected MSCs were further characterized for multilineage differentiation potential and the standard positive and negative surface markers of hBM-MSCs. We used the human granulosa cells (HGrC1) treated with 100 μg/ml of cyclophosphamide for 24 h as an in vitro POI model and treated these damaged HGrC1 cells with the secretome of miR144-5p overexpressing hBM-MSC for 24 hours post cytotoxicity. Cell proliferation (MTT assay) and the cell viability (XTT) between groups were studied. Relative gene expression was studied using three sets of genes which include i) markers of cell proliferation and survival (AKT, Ki67 and TK1) ii) markers of apoptosis (Cas3, Bcl-2 and Bax) iii) makers of steroidogenesis (StAR, CYP19A1 and FSHR). The secretome treated HGrC1 group showed improved cell proliferation and increased cell viability ($p< 0.05$) compared to untreated damaged cells. Among the genes tested, the relative gene expression of cas-3, a major executioner of apoptosis was downregulated ($p=0.01$) whereas Bcl-2, an anti-apoptotic marker in secretome treated group was significantly upregulated ($p< 0.001$) compared to untreated damaged cells. These preliminary findings encourage us to study the role of spe-

cific miR144-5p mediated anti-apoptotic pathways for the regenerative potential of genetically modified hBM-MSC which warrant as a novel therapeutic option for POI patients.

Funding Source: This study supported by start-up fund of the University of Chicago to Prof. Ayman Al-Hendy
Keywords: Premature Ovarian Insufficiency, microRNA 144-5p, Bone Marrow Mesenchymal Stem cells

TOPIC: NEURAL

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Stem Cell Mitochondrial Fitness to Enhance Neurotransplantation for Spinal Cord Injury

Zahedi, Atena - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Totounji, Iyah - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Khamnei, Neda - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Martinez, Joshua - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Wu, Anne - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Nadimzadeh, Narjes - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Lakatos, Anita - *Stem Cell Research Center, University of California, Irvine, CA, USA*
Anderson, Aileen - *Stem Cell Research Center, University of California, Irvine, CA, USA*

Abstract: Stem cells can contribute to neurorepair by generating new CNS cells and have shown to be promising in clinical therapies for traumatic injuries. However, neural regeneration after spinal cord injury (SCI) is hindered due to the harsh ischemic oxidative microenvironment, loss of mitochondria bioenergetics in the damaged tissues, and the presence of pro-inflammatory signals such as complement component C1q. We have previously derived and characterized several human neural stem cells (UCI-hNSCs) lines and their potential for restoring locomotor function (efficacy) after SCI in mice. We hypothesized that the survival and efficacy of donor stem cells in the SCI niche may depend on their mitochondria fitness traits (MFT): bioenergetics, biogenesis, permeability transition, redox potential, and mitophagy/autophagy. Transcriptomic comparison of an efficacious line (UCI161) vs. a non-efficacious line (UCI152) revealed key MTF differences. We have reported that C1q, which is present in the SCI inflammatory microenvironment, modulates hNSC fate, migration, and capacity for in vivo repair. Here we should that C1q exposure leads to mitochondria fission and generation of dysmorphic swollen mitochondria in UCI hNSC cultures. Consistent with RNA-seq data for these lines, C1q treatment for 2 days leads to loss of TFAM (biogenesis transcription factor) and activation of the inflammasome pathway. To test whether MFTs could be modulated pharmacologically, we screened and tested several drugs that are known to modulate mitochondrial function. Treatment of UCI161 hNSC with a bioenergetics-enhancing drug led to increased mitochondrial hyperfusion, whereas treatment with a biogenesis-enhancing drug led to increased mitochondria mass, cell yield and neuronal lineage selection following differentiation. Assays of metabolism, autophagy, mitochondria membrane potential (MMP), and ATP production revealed that UCI161 were better responders to bioenergetic drugs, whereas UCI152 responded optimally to biogenesis enhancement. Future studies will focus on mitochondria-based approaches to modulate the role of mitochondria fitness and mitochondria transfer in SCI to enhance the efficacy and behavior of transplanted hNSCs.

Keywords: Mitochondria Fitness, Neurotransplantation, Spinal Cord Injury

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A COMBINED CELL AND GENE THERAPY APPROACH FOR HOMOTOPIC RECONSTRUCTION OF MIDBRAIN DOPAMINE PATHWAYS USING HUMAN PLURIPOTENT STEM CELLS

Moriarty, Niamh - *Stem Cells & Neural Development Lab, The Florey Institute of Neuroscience and Mental Health, Parkville, Australia*

Abstract: The transplantation of midbrain dopaminergic (mDA) neurons can provide long term improvements in the motor function of Parkinson's Disease patients. Moreover, it is the capacity of these mDA neurons to form a functional terminal network with the host striatum that underpins their therapeutic efficacy. However, limited capacity for long-distance axonal growth within the adult brain requires cells to be transplanted ectopically, into the striatal target, rather than homotopically into their normal midbrain location. Consequently, several mDA pathways are not re-instated, which may be an underlying reason for the incomplete restoration of motor function seen in patients. Using a combined gene and cell therapy approach, we show that viral delivery of GDNF (Glial cell line-derived neurotrophic factor) to the target striatum, in conjunction with homotopic transplantation of human pluripotent stem cell derived mDA neurons to the midbrain, recapitulates brain-wide mDA target innervation. The grafts provided not only a re-instatement of striatal dopamine levels and correction of motor function comparable to ectopic grafts, but also connectivity with additional mDA target nuclei not well innervated by ectopic grafts. These results demonstrate the remarkable capacity for achieving functional and anatomically precise reconstruction of long-distance circuitry in the adult brain by matching appropriate growth-factor signalling to grafting of specific cell types.

Keywords: Parkinson's disease, Neural transplantation, GDNF

TOPIC: PLURIPOTENT STEM CELLS

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QUALITY ASSURED STEM CELLS: A JOURNEY TO CLINICAL APPLICATION

Mattioli, Elisa - *UKSCB, UKSCB/MHRA, Potters Bar, UK*
Konstantinidou, Chrysoula - *UKSCB, UKSCB/MHRA, London, UK*
Lindsay-Hill, Luke - *UKSCB, UKSCB/MHRA, London, UK*
Holmes, Richard - *UKSCB, UKSCB/MHRA, London, UK*
Prince, Judith - *UKSCB, UKSCB/MHRA, London, UK*
Warre-Cornish, Katherine - *UKSCB, UKSCB/MHRA, London, UK*
Perfect, Leo - *UKSCB, UKSCB/MHRA, London, UK*
Wise, Clare - *UKSCB, UKSCB/MHRA, London, UK*

Abstract: 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 for research and clinical applications. The UKSCB activities are carried out in purpose built, state-of-the-art facilities, which are licensed by the Human Tissue Authority (HTA) for clin-

ical use. Our repository consists of 183 cell lines from all over the world which are divided in 2 grades: research (145 cell lines) and clinical (38 cell lines). Since 2006, the UKSCB has distributed 320 cell line vials across many countries. The regenerative medicine field faces many challenges for the widespread adoption of cell therapies. Pluripotent stem cells offer unique potential for both standardisation and scalability of cell therapy starting materials. The UKSCB has developed a scrupulous QC testing strategy to assure critical qualities of pluripotent stem cell banks, including viability, sterility, identity and genomic integrity. We also measure parameters such as pluripotency and tri-lineage germ-layer differentiation and investigate the effects of processing variables using multivariate analysis. Furthermore, 28 clinical grade master cell banks have undergone additional deep characterisation, consisting of whole genome sequencing and deep sequencing of 400+ known oncogene loci. We are working in collaboration with the CJD Research and Resource Centre to develop a validated assay for the detection of transmissible spongiform encephalopathies (TSEs) suitable for clinical grade cell banks. In parallel, the UKSCB undertakes research to improve the standardisation, quality and safety of stem cell-based products. At the forefront, is our commitment to the WHO to develop International Standards and Reference Reagents for pluripotent and mesenchymal stromal cells. We are also using transcriptomic analysis to characterise cell therapy products, engaging in the development of an automated cell expansion system and developing a cell-based assay for the tetanus vaccine. The UKSCB is at the centre of the advanced therapy landscape and it is our key objective to supply high quality, ethically sourced, deeply characterised stem cell lines to support research and as starting materials for the development of cell-based therapies.

Keywords: Embryonic Stem Cells (ESCs), Advanced Therapy Medicinal Products (ATMPs), Quality Control Testing

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ALLOGENEIC IPSCS SURVIVE WITHOUT IMMUNOSUPPRESSION IN A TRANSLATIONAL NON-HUMAN PRIMATE MODEL

Schrepfer, Sonja - *Hypoimmune Platform, Sana Biotechnology Inc, San Mateo, CA, USA*
Hu, Xiaomeng - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
White, Kathy - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
DeJesus, Rowena - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Dominguez, Antonia - *Technical Sciences, Sana Biotechnology Inc, San Francisco, CA, USA*
Dowdle, William - *Technical Sciences, Sana Biotechnology Inc, San Francisco, CA, USA*
Olroyd, Ari - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Young, Chi - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Gattis, Corie - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Basco, Ron - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Chu, Elaine - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
Friera, Annabelle - *Hypoimmune Platform, Sana Biotechnology Inc, San Francisco, CA, USA*
McGill, Trevor - *Development Sciences, Sana Biotechnology Inc, San Francisco, CA, USA*
Deuse, Tobias - *Surgery, UCSF, San Mateo, CA, USA*

Abstract: Transplantation of cells that are allogeneic—derived from a donor who is not genetically identical to the recipient—induces an immune response that subsequently leads to rejection of the transplant. Approaches using HLA matching of banked donor cells with patients have a low likelihood for clinical success given that MHC-matched cell transplants in NHPs have consistently failed to prevent rejection. To avoid rejection absent immunosuppression (IS), rhesus macaque (NHP) iPSCs were engineered to knock out function of MHC class I and II and overexpress CD47 (HIP iPSCs). HIP iPSCs and wild type iPSCs (wt iPSCs) were transplanted into allogeneic, immunocompetent NHPs without IS and followed over time. At all time points tested up to 10 weeks, peripheral blood mononuclear cells killed wt iPSCs via direct cytotoxicity and antibody-mediated cellular cytotoxicity. There was a strong IFN-g ELISpot T cell response one week after wt iPSC transplantation, accompanied by a surge in wt iPSC specific IgM antibodies and, with delayed kinetics, also IgG antibodies. Furthermore, wt iPSCs were killed via complement-dependent and antibody-dependent cellular cytotoxicity. In contrast, NHPs that received HIP iPSCs showed no measurable immune response against HIP iPSCs at all time points. After 6 weeks, NHPs initially receiving wt iPSCs were injected with HIP iPSCs. Although they maintained their strong immune response against wt iPSCs, they did not mount any response against HIP iPSCs, indicating that HIP iPSCs evade immune recognition even in sensitized recipients. NHPs receiving HIP iPSCs first developed a strong cellular and antibody-response against the subsequently injected wt iPSCs but continued to have no reactivity against HIP iPSCs, demonstrating that the HIP iPSCs do not alter the recipient's immune system. Bioluminescence imaging up to 16 weeks revealed rejection of all wt iPSC grafts in both groups within 2-3 weeks after transplantation, while all HIP iPSC grafts survived the study period. In vitro characterization and mouse experiments demonstrated that HIP edits did not affect pluripotency of engineered iPSCs and that both wt and HIP iPSCs formed tissues of all 3 germ layers. HIP engineered cells hold promise to achieve long-term graft survival in patients leading to a new class of cell-based medicines.

Keywords: pluripotent stem cells, immune barrier, hypoimmune

222

CGMP COMPLIANT IPSC CELL GENOME EDITING PLATFORM FACILITATES ITS THERAPEUTIC APPLICATION

Tsai, Ruby - *Research and Development, Applied StemCell Inc., Milpitas, CA, USA*
Wang, Kai - *R&D, Applied StemCell, Inc., Milpitas, CA, USA*

Abstract: The advent of hiPSC technology (Human Induced Pluripotent Stem Cells) and genome editing technologies (e.g., CSRPR/CAS9 and TARGATT) allow iPSC and its derived functional cells to be broadly used in gene/cell therapy and regenerative medicine. High-quality standard, such as cGMP/cGMP-compliant, is necessary to ensure the success of genome-edited iPSC-based therapeutic products in both preclinic and clinic phases. However, building cGMP capabilities in this field is challenging and needs intense investment, quality management system, and an experienced scientific and manufacturing team. In response to the needs of clients and market trends, we have established cGMP manufacturing processes for iPSC reprogramming, gene editing

(CRISPR and TARGATT), cell banking, and iPSC differentiated products. Our cGMP facility is fully certified with a drug manufacturing license from the State of California Food and Drug Branch. For immune-oncology cell products, we have established a manufacturing process using TARGATT master iPSC platform for the development of CAR-iNK products. A TARGATT master iPSC line containing a landing pad in a safe harbor genomic locus has been established and used to insert a CD19-CAR (>6kb in size). The CAR insertion efficiency was over 40% without selection (10 times better than CRISPR/Cas9 method). The CAR-iPS cells were differentiated ex vivo to iNK cells. Quantitative PCR analysis indicated that CD19-CAR was expressed in all stages of cells during differentiation as well as in the final iNK cells. The same process is now being repeated in the GMP facility. In conclusion, our cGMP compliant iPSC capability provides a necessary resource for our clients who are developing therapeutics cell products, with a focs in CAR-iNK products.

Funding Source: N/A
Keywords: iPSCs, GMP, NK, Genome Editing, CAR T, HPCs, iPSCs, GMP, NK, Genome Editing, CAR T, HPCs, iPSCs, GMP, NK, Genome Editing, CAR T, HPCs

TOPIC: EPITHELIAL_LUNG

816

APPLICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED AIRWAY PROGENITORS FOR GENERATION OF LONG SEGMENT TRACHEAL GRAFTS

Karoubi, Golnaz - *Laboratory Medicine and Pathobiology, University Health Network, Toronto, ON, Canada*
Rostami, Sara - *Latner Thoracic Surgery Research Laboratories, University Health Network, Toronto, ON, Canada*
Marin-Araujo, Alba - *Latner Thoracic Surgery Research Laboratories, University Health Network, Toronto, ON, Canada*
Varma, Ratna - *Institute of Biomaterials and Biomedical Engineering, University of Toronto, ON, Canada*
Aoki, Fabio - *Departamento de Ciência e Tecnologia, Universidade Federal de São Paulo, Brazil*
Romero, David - *Department of Mechanical Engineering, University of Toronto, ON, Canada*
Amon, Cristina - *Department of Mechanical Engineering, University of Toronto, ON, Canada*
Waddell, Thomas - *Latner Thoracic Surgery Research Laboratories, University Health Network, Toronto, ON, Canada*
Haykal, Siba - *Latner Thoracic Surgery Research Laboratories, University Health Network, Toronto, ON, Canada*

Abstract: Long-segment airway stenosis and disease require transplantation, which fails due to the epithelial dysfunction and immune rejection. Tissue engineered tracheal grafts are promising however repopulation of fully decellularized tracheal scaffolds remains challenging. We have developed a bioreactor-based partial decellularization protocol for pig tracheae which removes the epithelium while keeping the immune-privileged cartilage alive. We differentiated human induced pluripotent stem cells (hiPSCs) to generate proximal airway progenitor cells (APs) and used hiPSC-derived APs to repopulate de-epithelialized long segment grafts in a biomimetic bioreactor system which allows for fully submerged and air-liquid interface (ALI) culture. Cells were seeded at 1.0x106 cells/cm2 and grafts were evaluated after 3, 10 and 17 days in the bioreactor. Cell viability analysis was done using a Live/Dead stain and differentiation was assessed using immunofluorescence staining for P63, Keratin 5 (KRT5), Keratin 8 (KRT8), Mucin 5AC (MUC5AC) and acetylated tubulin. Using the NKX2.1+ reporter BU3NG hiPSC line, we generated 38.1±3.9% GFP-NKX2.1+ day 15 lung progenitors which were further cultured and expanded in 3D Matrigel conditions to give rise to 65.6±2.4% P63+ airway progenitor (AP) cells on day 42. The APs were then seeded onto 5-cm long de-epithelialized tracheal grafts in the bioreactor using bidirectional perfusion cell seeding, followed by unidirectional flow at 1.5ml/min and 1 rpm for 3 days in the bioreactor at 1x106 cells/cm2 under submerged culture. Results demonstrated uniform cell coverage of the tracheal graft and formation of a monolayer along the tracheal lumen at 3 days. The attached cells were P63-KRT5+KRT8+, indicating an intermediate cell differentiation stage. An additional 7 and 14 days under ALI conditions resulted in differentiation of APs to MUC5AC expressing goblet cells and the appearance of acetylated tubulin expressing ciliated cells. Future studies will include proof-of-concept orthotopic transplantation in a preclinical model. The development of non-immunogenic chimeric tracheal grafts with recipient-derived epithelium will have significant implications in the field of tracheal transplantation.

Funding Source: Research supported by s Canada First Research Excellence Fund (Medicine by Design) and the Canadian Donation and Transplantation Research Program
Keywords: Tracheal Regeneration, Airway Progenitors, Pluripotent Stem Cells

TOPIC: GERMLINE

818

GENERATION OF HUMAN IPS CELL-DERIVED LEYDIG CELLS AND THEIR ENCAPSULATION IN IMPLANTABLE DEVICES

Sato, Katsuya - *Department of iPS cell Applications, Kobe University Graduate School of Medicine, Kobe, Japan*
Koyanagi-Aoi, Michiyo - *Department of iPS cell Applications, Kobe University Graduate School of Medicine, Kobe, Japan*
Fujisawa, Masato - *Division of Urology, Kobe University Graduate School of Medicine, Kobe, Japan*
Aoi, Takashi - *Department of iPS cell Applications, Kobe University Graduate School of Medicine, Kobe, Japan*

Abstract: Late-onset hypogonadism (LOH), caused by a decrease in serum testosterone secreted by Leydig cells, has been attracting attention. In the past, we had successfully induced Leydig cells from human iPS cells by forcibly expressing NR5A1, a gene necessary for Leydig cell differentiation. However, the forced expression of NR5A1 was performed using the Tet-On system, which required the continuous addition of doxycycline to keep the system running, making it unsuitable for clinical application. In addition, for in vivo transplantation experiments, it is necessary to protect the generated Leydig cells from the attack of the recipient's immune cells. To generate Leydig cells from human iPS cells without depending on doxycycline by creating the NR5A1 constant expression system. In addition, to realize the secretion of testosterone into the culture supernatant while the generated Leydig cells are encapsulated in the implantable device. A plasmid for the constant expression of NR5A1 was transfected into human iPS cells, and the cells were differentiated into Leydig cells via the embryoid body. The cells were encapsulated in an implantable device made from alginate, and culture was contin-

ued to evaluate the testosterone concentration in the supernatant. We have successfully generated Leydig cells that constantly express NR5A1 and produce testosterone without depending on doxycycline. We have also confirmed that the cells secrete testosterone even when encapsulated in an implantable device. It is no longer necessary to continue adding doxycycline to induce differentiation of Leydig cells as in the past. The cells were also able to secrete testosterone after being encapsulated in an implantable device for in vivo transplantation. Thus, we can say that we have made steady progress toward transplantation for patients with Male hypogonadism.

Keywords: Leydig cell, differentiation, iPSC

TOPIC: LIVER

820

EFFECTS OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS ON MITOCHONDRIAL OXIDATIVE DYSFUNCTION IN NON-ALCOHOLIC FATTY LIVER DISEASE

Park, Min Kyung - *Department of Internal Medicine and Liver Research Institute, Seoul National University, Seoul, Korea*
Lee, Yun Bin - *Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea*
Jung, Se-Mi - *Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea*
Kwon, Yoo-Wook - *Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea*
Yoon, Jung-Hwan - *Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea*

Abstract: Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide, but its pathophysiology is not fully understood due to the complexity of the mechanisms involved in the disease. Moreover, pharmacological therapy for NAFLD is not yet available. We investigated the therapeutic potential of induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSCs) on hepatic steatosis and mitochondrial oxidative function. HepG2 cells were treated with palmitic acid (PA) and then co-cultured with iPS-MSCs. Intracellular lipid accumulation was measured by oil red O staining. The mitochondrial oxidative function was assessed by quantifying mitochondrial mass and measuring reactive oxygen species (ROS) and the activity of antioxidant enzymes. C57BL/6 mice were chronically fed with choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD). At week 20, mice were injected with either phosphate-buffered saline or iPS-MSCs (1,000,000 cells). Four weeks later, liver histology and function were assessed. PA-induced intracellular lipid accumulation was attenuated when co-cultured with iPS-MSCs. The mitochondrial mass was reduced by PA treatment and then was restored following co-culture with iPS-MSCs. Increased cellular ROS production by PA treatment was attenuated after co-culture with iPS-MSCs. The activity of superoxide dismutases (SODs) and the ratio of reduced/oxidized glutathione were decreased by PA treatment and were restored by co-culture with iPS-MSCs. After infusion of iPS-MSCs, successful engraftment of transplanted stem cells was confirmed, leading to amelioration of severe hepatic steatosis in CDAHFD-fed mice. Decreased mitochondrial DNA content in CDAHFD-fed mice was reinstated to near-control level after transplantation of iPS-MSCs. Augmented hepatic ROS accumulation caused by CDAHFD was attenuated after transplantation of iPS-MSCs with dynamic changes in the activity of SODs and the ratio of glutathione. Hepatic steatosis and mitochondrial oxidative dysfunction in NAFLD can be ameliorated by transplantation of iPS-MSCs. Our study findings suggest the therapeutic potential of iPS-MSCs in NAFLD and help understanding alterations in hepatic lipid metabolism, which may be restored by iPS-MSC transplantation.

Funding Source: This study was supported by research grants from the Yuhan Pharmaceuticals (grant no. 06-2020-2900).
Keywords: Human induced pluripotent stem cell, Nonalcoholic fatty liver disease, Mitochondrial oxidative dysfunction

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

822

IMPROVING THE SURVIVABILITY OF EQUINE MESENCHYMAL STROMAL CELLS IN A SERUM FREE CULTURE

Pilgrim, Cara - *Biomedical Science, University of Guelph, ON, Canada*
Oji, Saba - *Biomedical Science, University of Guelph, ON, Canada*
Koch, Thomas - *Biomedical Science, University of Guelph, ON, Canada*
Madan, Pavneesh - *Biomedical Science, University of Guelph, ON, Canada*

Abstract: Equine mesenchymal stromal cells (MSCs) have become an important tool in mitigating orthopedic injuries. In order to use these cells for such therapeutic interventions, MSCs are generally cultured in serum (FBS) supplemented media. However, FBS supplementation raises concerns with sourcing, batch to batch variation, and possible immunogenic mismatch. The use of commercially available serum-free media (SFM) is an alternative but equine MSCs have shown reduction in proliferation and functionality in this media as it is more tailored to human MSCs. Recent studies in our lab used spent media metabolomic analysis to show that various pathways significantly differed between equine cord blood MSCs (eCB-MSCs) serum and serum free conditions. Most drastically, MSCs cultured in serum-free media lacked the expression of taurine and hypotaurine as compared to cells cultured with FBS. MSCs cultured in SFM show altered morphology and a significant increase in population doubling time (P=0.02) when compared to MSCs grown in standard serum conditions. Through mass spectrometry analysis we confirmed that while cells cultured in media containing serum had taurine present in the intracellular environment, cells cultured in SFM did not. Further investigation of the Taurine and Hypotaurine pathway by qPCR revealed a significant increase (p=0.03) in Cystine Sulfinic Acid Decarboxylase (CSAD), the rate limiting enzyme in the Taurine biosynthetic pathway within MSCs grown in SFM compared to serum media. When these cells were supplemented with 5mM of taurine into the SFM, a correction in CSAD expression was found (P=0.02). This expression level was significantly lower than expression in MSCs grown in SFM and at a comparable level to MSCs cultured in serum media. Despite the correction in gene expression, there was no change in the population doubling time or the morphology of these cells. This indicates that supplementing SFM with 5mM of taurine can correct changes in gene expression

associated with the taurine and hypotaurine pathway. However, this seems to not be the only supplement required to support the growth of eCB-MSCs in SFM. Therefore, further supplements need to be evaluated to tailor StemPro SFM to the culture of eCB-MSCs. This will create a safer and more consistent culture condition for culturing MSCs in vitro.

Funding Source: This research was supported by Ontario Veterinary College Scholarship and NSERC
Keywords: Serum Free Media, Mesenchymal Stromal Cells, Metabolomics

TOPIC: CARDIAC

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EPICARDIALLY-SECRETED FIBRONECTIN DRIVES CARDIOMYOCYTE MATURATION IN 3D-ENGINEERED HEART TISSUES

Ong, Lay Ping - *Medicine - Cardiology, University of Cambridge, UK*
Bargehr, Johannes - *Medicine, University of Cambridge, UK*
Knight-Schrijver, Vincent - *Medicine, University of Cambridge, UK*
Lee, Jonathan - *Medicine, University of Cambridge, UK*
Colzani, Maria - *Medicine, University of Cambridge, UK*
Bayraktar, Semih - *Medicine, University of Cambridge, UK*
Bernard, Will - *Medicine, University of Cambridge, UK*
Marchiano, Silvia - *Pathology, University of Washington, Seattle, WA, USA*
Bertero, Alessandro - *Molecular Biotechnology and Health Sciences, University of Torino, Italy*
Gambardella, Laure - *Cellular Genetics, Wellcome Trust Sanger Institute, Cambridge, UK*
Sinha, Sanjay - *Medicine, University of Cambridge, UK*

Abstract: Epicardial-myocardial crosstalk underpins key events during cardiac embryogenesis and epicardial reactivation following cardiac injury. However, the exact paracrine signals utilised in epicardial-myocardial crosstalk remain unclear. Recently, we showed that co-culture of human embryonic stem cells (hESC)-derived epicardium in 3D-engineered heart tissues (3D-EHTs) promoted hESC-cardiomyocyte maturation. Fibronectin (FN) was uniquely upregulated in 3D-EHTs co-cultured with hESC-epicardium, compared to other stromal cells such as mesenchymal stem cells. Thus, we hypothesised that epicardial-secreted fibronectin is a key mediator of the epicardial-myocardial crosstalk leading to cardiomyocyte maturation. To test this hypothesis, we performed a series of loss of FN function experiments in 3D-engineered heart tissues (3D-EHTs). We used the recombinant inhibitory peptide pUR4 (1), then a Crispr-Cas9 mediated knockout (KO) of FN in hESC (2) and finally a tetracycline-inducible FN-KO hESC line (sOPTiKD-FN) (3). We demonstrated that the loss of epicardial-FN, at both protein and gene level, impaired cardiomyocyte structure and function with decreased downstream FN-integrin α5β1 clustering. Furthermore, loss of epicardial-FN impaired active force generation and Ca2+-kinetics in 3D-EHTs whilst preserving passive force. Using RNA bulk sequencing data, we elucidated the epicardium-cardiomyocyte crosstalk and the role of FN within, revealing a signalling network of myocardial growth and regeneration. In conclusion, epicardial-fibronectin is a key mediator of epicardial-cardiomyocyte crosstalk to drive hESC-cardiomyocytes' maturation in 3D-EHTs. Loss of FN at different stages of hESC-epicardium differentiation and maturity consistently impaired 3D-EHTs' cardiac function. Our regulatory gene network of epicardial-myocardial crosstalk highlights the crucial role of FN and offers mechanistic insight into key developmental pathways for further studies.

Funding Source: Wellcome Trust, Addenbrooke's Charitable Trust
Keywords: epicardium, cardiomyocyte maturation, 3D-engineered heart tissues

TOPIC: PLURIPOTENT STEMCELL

882

MULTI-TISSUE MODELING OF BRCA1/2 CANCERS USING IPSC-DERIVED 3D HUMAN ORGANOID PLATFORM

Yucer, Nur - *RMI, Cedars-Sinai Health System, Los Angeles, CA, USA*
Chiou, Janice - *Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Plummer, Jasmine - *Center for Bioinformatics and Functional Genomics, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ellis, Leigh - *Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Cui, Xiaojiang - *Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Gayther, Simon - *Department of Biomedical Sciences,, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Svendsen, Clive - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Germline mutations in BRCA1 and BRCA2 (BRCA1/2) genes are the strongest genetic risk for familial ovarian and breast cancer. Pathogenic variants in these genes are implicated in ~15% of women with heritable risks of these cancers. BRCA1/2 mutations are also associated with risk of pancreatic cancers in men and women. Precise risk estimates for pathogenic variants in BRCA1/2 affecting different cancer types are critical to assess treatment options and improve drug sensitivity. Yet, associations of pathogenic variants between BRCA1/2 and cancer types are poorly understood, and mechanistic studies are limited by a lack of reliable models. Patient-specific induced pluripotent stem cell (iPSC) and 3D-tissue engineering technologies can now be used to model human disease. iPSCs from patients with known genetic mutations will harbor the disease mutation and the patient's genetics. Several iPSC-derived, inherited disease models have reproduced associated high-risk cancers and revealed disease pathogenesis and carcinogenesis in relevant cells. This study explores effects of BRCA1/2 mutations on early-stage genomic alterations associated with cancer development and progression. We generated iPSCs from BRCA1mut female patients with ovarian cancer and are now generating iPSCs from female and male BRCA2mut carriers. We have generated iPSC-derived organoid models of ovarian cancer (fallopian tube epithelium, FTE) and breast cancer (mammary gland epithelium, MGE) from female BRCA1mut carriers and generated prostate cancer precursors from BRCA2mut carriers. After differentiation into FTE organoids, BRCA1mut lines exhibit cellular abnormalities similar to neoplastic transformation with expression of cancer-specific biomarkers, and various structural abnormalities. Organoids from women with the most aggressive ovarian cancer showed the most severe neoplastic pathology, suggesting this may be a patient-specific predictive model. We have extended studies to generate MGE

organoids in the same BRCA1mut lines and to develop FTE, MGE, and/or prostate organoids from female and male BRCA2mut carriers. These iPSC-derived multi-tissue organoids provide a physiological model that recapitulates BRCA1/2 cancer precursor tissues, allowing cancers to evolve in vitro for mechanistic and drug screening studies.

Funding Source: Regenerative Medicine Institute at Cedars-Sinai, the Ovarian Cancer Research Fund, Ann and Sol Schreiber Mentored Investigator Award, Veterans Administration Merit Award, Department of Defense Breakthrough Award
Keywords: BRCA1/2 Cancer Modeling, Organoids, iPSC

POSTER SESSION II: EVEN

4:00 PM – 5:00 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

302

THE ROLE OF YES ASSOCIATED PROTEIN (YAP) IN HYPERTROPHIC CARDIOMYOPATHY

Chirikian, Orlando - *Biomolecular Science and Engineering, University of California, Santa Barbara, CA, USA*
Faynus, Mohamed - *Biomolecular Science and Engineering, University of California, Santa Barbara, CA, USA*
Merk, Markus - *Biomolecular Science and Engineering, University of California, Santa Barbara, CA, USA*
Singh, Zach - *Molecular Cellular and Developmental Biology, University of California, Santa Barbara, CA, USA*
Muray, Christopher - *Physics, University of California, Santa Barbara, CA, USA*
Pham, Jeffrey - *Molecular Cellular and Developmental Biology, University of California, Santa Barbara, CA, USA*
Pruitt, Beth - *Biomolecular Science and Engineering, University of California, Santa Barbara, CA, USA*

Abstract: Hypertrophy Cardiomyopathy (HCM) is the most prevalent hereditary cardiovascular disease – affecting 1 in 500 individuals. Advanced forms of the disease clinically present with hypercontractility, hypertrophy and fibrosis. Several single-point mutations in b-myosin heavy chain, Myosin Binding Protein C, and Troponin have been associated with HCM and increased contractility at the organ level. However, the kinetics at the molecular level remain unclear, as different sarcomeric protein mutations can result in increased, decreased, or unchanged force production. A knowledge gap persist in understanding how these altered kinetics at the molecular level lead to the more advanced hypertrophic phenotype of HCM at the cellular level. Interestingly, the Hippo Pathway has been demonstrated to be activated during developmental growth, quiescent during cardiac homeostasis, and reactivated in pathological growth (i.e. HCM). However its involvement in the disease, in particular the initiation of the hypertrophic phenotype, is poorly understood. Here, we aim to understand whether homeostatic mechanical signaling through the canonical growth regulator, Hippo-YAP, is altered 1) by changes in the biomechanics of single HCM mutant cardiomyocytes and 2) by alterations in the mechanical environment. We use hiPSC-CMs genetically edited to harbor point mutations associated with HCM, as a reduce ordered model to study the relationship between mechanical signaling and hypertrophic growth. To further elucidate the mechanism by which YAP is contributing to the phenotypes of HCM we have developed a novel optogenetic tool, termed OptoYAP, which provides full temporal and spatial control of the Hippo pathway. Lastly we aim to understand the mechanism behind the reactivation of YAP in pathological conditions by perturbing the mechanical signaling by the nucleus. We hypothesize that 1) changes in force production alter the homeostatic mechano-signaling of the Hippo pathway to initiate cellular hypertrophy and 2) subsequent changes to the extracellular environment (stiffening) compounds this effect leading to a feedforward signal progressing the disease phenotypes. 3) pathological YAP signaling is driven by excessive force transmission by the cytoskeleton resulting in nuclear deformation.

Keywords: Stem Cells, Cardiomyocytes, Hypertrophic Cardiomyopathy

304

ROBUST AND EFFICIENT GENERATION OF FUNCTIONAL HUMAN PLURIPOTENT STEM CELL-DERIVED ATRIAL CARDIOMYOCYTES

Legree, Jessica C. - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Ang, Trisha - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Nguyen, Vivian - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Luo, Hattie - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Szilvassy, Steve - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Corporate, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Macri, Vincenzo - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: The ability to generate and maintain high-quality human pluripotent stem cell (hPSC)-derived atrial cardiomyocytes is necessary to model atrial cardiomyocyte disease and for drug discovery and toxicology. Protocols and reagents to make hPSC-derived atrial cardiomyocytes are variable and not standardized. We have developed the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit, a defined, serum-free cell culture medium to differentiate hPSCs to atrial cardiomyocytes. hPSCs were seeded as single cells in TeSR™ medium containing Y-27632 and maintained for 2 days. Differentiation of hPSCs was initiated with a full-medium change using the STEMdiff™ Atrial Cardiomyocyte Differentiation Kit. After 15 days, a confluent, beating monolayer was observed that contained > 80% cardiac troponin T-positive cells, as determined by flow cytometry. Quantitative PCR indicated that hPSC-derived atrial cardiomyocytes have significantly increased expression of KCNJ3, an atrial-specific potassium channel gene (> 30-fold, n = 4 hPSC lines), and significantly decreased expression of MYL2, a ventricular-specific myofilament gene (< 0.3-fold, n = 4 hPSC lines), when compared to hPSC-derived ventricular cardiomyocytes. hPSC-derived ventricular cardiomyocytes were generated with the STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. RNA sequencing showed upregulation of atrial-specific genes (KCNJ3, NPPA, NR2F2, PITX2) and downregulation of ventricular-specific genes (MYL2, MYH7, HEY2, HAND1) for hPSC-derived atrial cardiomyocytes compared to hPSC-derived ventricular car-

diomyocytes. Microelectrode array electrophysiology indicated that hPSC-derived atrial cardiomyocytes had a shorter field potential duration and increased beat rate compared to hPSC-derived ventricular cardiomyocytes. The STEMdiff™ Atrial Cardiomyocyte Differentiation Kit is a standardized culture system to efficiently generate functional hPSC-derived atrial cardiomyocytes that can be used to model atrial disease and perform drug discovery and toxicology studies.

Keywords: Atrial cardiomyocytes, Differentiation, Cardiac

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MODELING LMNA-RELATED CARDIOMYOPATHY USING PATIENT-SPECIFIC HUMAN INDUCED PLURIPOTENT STEM CELLS

Fonoudi, Hananeh - *Pharmacology, Northwestern University, Chicago, IL, USA*
Kuo, Hui-Hsuan - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Jouni, Mariam - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Negahi Shirazi, Ali - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Gao, Xiaozhi - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Vanoye, Carlos - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Wilcox, Jane - *Bluhm Cardiovascular Centre of Northwestern, Northwestern Memorial Hospital, Chicago, IL, USA*
George, Alfred - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Burridge, Paul - *Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*

Abstract: Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy and is a leading cause of heart failure, arrhythmias, and heart transplantation. Thus far, more than 50 genes have been associated with DCM, from which LMNA is ranked the most highly associated, representing 5-10% of the cases. Currently, in many cases of LMNA-related DCM (LMNA-DCM), the direct link between the LMNA variants and DCM is not fully understood. Furthermore, there are no pharmaceutical therapeutics designed specifically for LMNA-DCM patients. In this study, we have generated an in vitro model of LMNA-DCM using patient-specific human induced pluripotent stem cells (hiPSCs). Eight individuals harboring four different pathogenic LMNA variations were selected. All LMNA variant carriers were symptomatic, showing arrhythmia and DCM. Three of the participants had undergone heart transplantation. Moreover, five healthy individuals were recruited as controls. hiPSCs were generated from all the individuals, then differentiated into cardiomyocytes and assessed at day 30 post differentiation. Cardiomyocytes derived from LMNA patients (LMNA-CMs) showed a significantly higher level of nuclear deformation compared to controls. Moreover, after two days of mechanical stress, LMNA-CMs showed significantly higher levels of nuclear dysmorphism while the controls were not affected. Analysis of the calcium (Ca2+) transient revealed prolongation of Ca2+ peaks and arrhythmia in LMNA-CMs. Additionally, contractility analysis using a multielectrode array revealed significantly lower beat rate and higher pulse width in LMNA-CM compared to controls. Previous studies have suggested that rapamycin (sirolimus), an mTOR inhibitor, might have a positive effect on mouse models of LMNA-DCM. To assess the effect of rapamycin treatment on human cells, LMNA-CM were treated with rapamycin for 3 days. Treatment of LMNA-CMs with rapamycin led to the correction of Ca2+ transient and contraction abnormalities. In summary, our finding thus far shows significant electrophysiological differences between LMNA-CMs and controls which could help to unravel the cellular mechanism underlying formation of arrythmia in LMNA-DCM patients and develop more effective treatments.

Funding Source: Hananeh Fonoudi is covered by American Heart Association Postdoctoral Fellowship.
Keywords: Cardiomyopathy, Disease modeling, Human Induced Pluripotent Stem Cells

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INHIBITION OF TBL1-CLEAVAGE REDUCES DOXORUBICIN-INDUCED APOPTOSIS IN CARDIOMYOCYTE

Lee, Sun-Ho - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Choi, Hyo-Kyung - *Division of Nutrition and Metabolism Research Group, Korea Food Research Institute, Seoul, Korea*
Oh, Jaewon - *Division of Cardiology, Severance Cardiovascular Hospital, Cardiovascular Research Institute, Seoul, Korea*
Kim, Hyeong-Jin - *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Park, So Hee - *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Yoon, Ho-Geun - *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Kang, Seok-Min - *Division of Cardiology, Severance Cardiovascular Hospital, Cardiovascular Research Institute, Seoul, Korea*
Park, Sahng Wook - *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Lee, Seung-Hyun - *Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Doxorubicin is a commonly used anthracycline cancer chemotherapeutic. Although its cardiotoxicity limits its clinical usefulness, the exact mechanism underlying this phenomenon remains to be elucidated. Transducin β-like protein 1 (TBL1) and TBLR1 (TBL1-related), two highly related F-box/WD-40 containing factors, were initially identified as components of the co-repressor SMRT/N-CoR complex. Previously, Mahrus et al. reported that SMRT/N-CoR complex components such as HDAC7 and TBLR1, are targets of caspase proteolysis during apoptosis. Also, Daniels et al. reported that TBLR1 N-term cleavage reduces apoptosis in prostate cancer under androgen deprivation. However, despite high homology between TBL1 and TBLR1, it is uncertain whether TBL1 is cleaved during apoptosis. So, we investigated whether cleavage of TBL1 and its function during apoptosis. To evaluate the predictable caspase-3 cleavage location of TBL1 protein, we first performed screening using CaspDB database in silico. In TBL1 protein, 11 predicted cleavable sites were addressed, and we performed an in vitro caspase cleavage assay. It showed that caspase 3, 7 dominantly made N-terminus cleavage. Also, in H9C2 rat myoblast cell, endogenous N-terminus TBL1 cleavage during doxorubicin-induced apoptosis was significantly increased in a time-dependent manner. Then, we investigated how apoptosis signaling changes when the cleavage of TBL1 is prevented. We found that non-cleavable TBL1 efficiently blocks apoptosis progression in the H9C2 cell line, but not in the U2OS osteosarcoma cell line. Next, to evaluate the functional effects of non-cleavable TBL1 protein, we performed Multi-electrode array

(MEA) analysis and calcium transient assay with human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. The MEA data analysis showed significant increases in the beat period, conduction velocity with non-cleavable TBL1 compared to wild-type TBL1 hiPSC-CMs and showed decreases in the beat period irregularity, spike slope, and spike amplitude. Also, the calcium transient assay data showed similar results to the MEA data analysis. These findings demonstrate that TBL1 could be a novel target for reducing doxorubicin-induced cardiotoxicity.

Funding Source: This work has supported by the Brain Korea 21 Project for Medical Science, Yonsei University, and the National Research Foundation of Korea (NRF) grant (2022R1H1A200391911, 2019R1C1C1002334).
Keywords: hiPSC-derived cardiomyocyte, TBL1, Dilated cardiomyopathy

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HUMAN IPSC-DERIVED CARDIOMYOCYTES AS AN IN VITRO MODEL FOR SARS-COV-2 INFECTION

Yankaskas, Chris - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Holmes, Spencer - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Kennedy, Mark - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*
Kuninger, David - *Cell Biology, Thermo Fisher Scientific, Frederick, MD, USA*

Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes cardiac symptoms in 20-44% of patients. Yet, the mechanism by which SARS-CoV-2 damages cardiac cells is poorly understood, and the long-term effects on the patient are largely unknown. Human induced pluripotent stem cells (iPSCs) can be differentiated to cardiomyocytes – the major cellular constituent of cardiac muscle – with a relatively high degree of purity for studying cellular function and disease modeling. iPSC-derived cardiomyocytes express angiotensin-converting enzyme 2 (ACE2) and were infected with SARS-CoV-2 spike protein pseudotyped lentivirus in a Biosafety Level (BSL) 2 setting. Addition of an anti-SARS-CoV-2 spike protein antibody significantly reduced the number of infected cells, indicating that available spike protein was required for this infection (presumably to interact with ACE2). In a BSL-3 setting, iPSC-derived cardiomyocytes were productively infected with live SARS-CoV-2 from the Washington and Delta strains. Both virus strains induced significant cytopathic effect in iPSC-derived cardiomyocytes. Taken together, these results demonstrate the utility of iPSC-derived cardiomyocytes for studying SARS-CoV-2 cardiac tropism and have the potential for use in the identification of cardioprotective therapeutics.

Keywords: Cardiomyocytes, SARS-CoV-2, COVID-19

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CELLULAR MODEL OF LMNA GENETIC DEFECTS LEADING TO HYPERTROPHIC CARDIOMYOPATHY

Dobrovolny, Robert - *First Faculty of Medicine, Research Unit for Rare Diseases, Charles University, Prague, Czech Republic*
Asfaw, Befekadu - *First Faculty of Medicine, Research Unit for Rare diseases, Charles University, Prague, Czech Republic*
Kubanek, Milos - *Department of Cardiology, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic*
Melenovská, Petra - *First Faculty of Medicine, Research Unit for Rare diseases, Charles University, Prague, Czech Republic*
Piherova, Lenka - *First Faculty of Medicine, Research Unit for Rare diseases, Charles University, Prague, Czech Republic*

Abstract: Cardiomyopathy is a disease of the heart muscle associated with a disorder of its function. This is a heterogeneous group of diseases with various clinical signs that can ultimately lead to heart failure. Subset of cardiomyopathies are genetically conditioned. iPSC derived from patients or engineered with specific genetic defects found in patients serve as a unique model of the disease for pathogenetic studies. We have focused on a group of cardiomyopathies with defect in lamin A/C gene (LMNA). Lamin A/C is a protein targeted to the nuclear membrane by an isoprenyl group and further processed. The protein defect then leads to various diseases like progeria, muscular dystrophy and heart malfunctions. We have produced three iPSC lines from patient with various mutations and clinical outcomes. The iPSC lines were than differentiated to cardiomyocytes and subjected to standard characterization such as differentiation efficiency, response to isoprenylation inhibitors, morphological characterization of nuclei. We have observed decreased differentiation efficiency of patient derived lines. The cardiomyocytes were morphologically different with abnormal nuclei. The isoprenylation inhibitor had limited effect on recovering the phenotype.

Funding Source: The project was supported by research grant from Agency for Medical Research of Czech Ministry of Health, reg. number NV19-08-00122.
Keywords: Cardiomyopathy, Lamin A/C LMNA, induced pluripotent stem cells iPSC

TOPIC: EARLY EMBRYO

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SINGLE-CELL ANALYSIS OF EMBRYOIDS REVEALS LINEAGE DIVERSIFICATION ROADMAPS OF EARLY HUMAN DEVELOPMENT

Fu, Jianping - *Mechanical Engineering, Biomedical Engineering, Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Zheng, Yi - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Yan, Robin - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Kobayashi, Mutsumi - *Massachusetts General Hospital Center for Cancer Research, Charlestown, MA, USA*
Xiang, Lifeng - *Department of Reproductive Medicine, the First People's Hospital of Yunnan Province, Kunming, China*
Yang, Ran - *Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*

Goedel, Alexander - *Department of Cell and Molecular Biology, Karolinska Institutet, Karolinska Institutet, Sweden*
Kang, Yu - *Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, China*
Xue, Xuefeng - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Esfahani, Sajedeh - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Liu, Yue - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Irizarry, Agnes - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Wu, Weisheng - *Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Li, Yunxiu - *Department of Reproductive Medicine, the First People's Hospital of Yunnan Province, Kunming, China*
Ji, Weizhi - *Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, China*
Niu, Yuyu - *Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, China*
Chien, Kenneth - *Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*
Li, Tianqing - *Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, China*
Shioda, Toshihiro - *Massachusetts General Hospital Center for Cancer Research, Charlestown, MA, USA*

Abstract: Despite its clinical and basic importance, our understanding of early human development remains limited. Stem cell-derived, embryo-like structures (or embryoids) allowing studies of embryonic development without using natural embryos can potentially help fill the knowledge gap of human development. Herein, transcriptome at the single-cell level of a human embryoid model, which recapitulates aspects of lineage diversification and three-dimensional tissue architecture of the human embryo from the implantation to the onset of gastrulation, was profiled at different time points. Molecular maps of lineage diversifications from the pluripotent human epiblast towards the amniotic ectoderm, primitive streak / mesoderm, and primordial germ cells were constructed and compared with in vivo primate data. Similarly, chimpanzee embryoids were generated and profiled to reveal transcriptome dynamics during the early post-implantation chimpanzee development. Our comparative transcriptome analyses reveal a critical role of NODAL signaling in human mesoderm and primordial germ cell specification, which is further functionally validated. Through comparative transcriptome analyses and validations with human blastocysts and in vitro cultured cynomolgus embryos, we further proposed stringent criteria for distinguishing between human trophectoderm and amniotic ectoderm cells. Altogether, this study provides new knowledge of the lineage diversification roadmap of early human development and will serve as a valuable resource for studying human development.

Keywords: Human embryoid, single-cell transcriptome, amnion

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MODELING NEURAL CREST DEFECTS IN DIGEORGE SYNDROME USING INDUCED PLURIPOTENT STEM CELLS

Berecz, Tunde - *National Institute of Dental and Craniofacial Research, National Institutes of Health, Rockville, MD, USA*
Apati, Agota - *Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary*
Kerosuo, Laura - *National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*

Abstract: Human disease models are crucial for understanding species-specific mechanisms, particularly when there is no appropriate animal model, or when human tissue samples are not available. In a human induced pluripotent stem cell (hiPSC)-based in vitro system, we model DiGeorge syndrome, a congenital developmental disorder that affects development of the cardiovascular, nervous and immune systems, as well as the craniofacial skeleton. As the most common microdeletion syndrome, it is caused by a heterozygous deletion on 22q11.2 chromosome region spanning approximately 50 protein coding, and 90 genes in total. Surprisingly, despite the same genetic deletion, DiGeorge syndrome patients manifest a diverse range of symptoms from mild to severe. Many of the DiGeorge syndrome symptoms are caused by defects in neural crest derived tissues. The neural crest is a transient stem cell population that rises from the neural plate border and migrates to various locations in the developing embryo and contributes to the formation of multiple tissues including bones and cartilage of the face, and also the cardiac outflow tract. Here, we address the role of neural crest defects in the development of DiGeorge syndrome. In the family involved in our study, we have three patients manifesting the disease with different severity and two healthy controls, giving us the unique opportunity to also study intrafamilial variability. We established hiPSC lines from all family members and differentiated them into neural crest cells (hiPSC-NCCs) by using an ectodermal organoid method that mimics in vivo steps of neural crest development in 3D. Our preliminary results indicate the patient cells have an impaired capacity to form neural crest already at the premigratory stage. Furthermore, ongoing investigations address whether also migration is impacted, and if the DiGeorge patient cells are able to properly differentiate into mesenchymal neural crest derived cells including chondrocytes, osteoblasts and vascular smooth muscle cells. In summary, our research gives insight into the cellular and molecular mechanisms underlying DiGeorge syndrome, and helps understand how these molecular events are linked to the severity of the disease, and also elucidate the role of environmental and epigenetic factors in the process.

Funding Source: Founded by: NIH DE000748-04 and NKFIH K128369
Keywords: DiGeorge syndrome, induced pluripotent stem cells, neural crest

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EXPLORING THE EARLY PATTERNING OF THE HUMAN EMBRYONIC BRAIN USING THE MISTR IN VITRO MICROFLUIDIC-CONTROLLED SYSTEM

Rifes, Pedro - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) / Department of Neuroscience, University of Copenhagen, Denmark*
Isaksson, Marc - *Department of Biomedical Engineering / Wallenberg Center for Molecular Medicine, University of Lund, Sweden*
Rathore, Gaurav - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) / Department of Neuroscience, University of Copenhagen, Denmark*
Ankjaer, Matias - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) / Department of Neuroscience, University of Copenhagen, Denmark*
Baungaard, Sara - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) / Department of Neuroscience, University of*

Copenhagen, Denmark
Kirkeby, Agnete - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) / Department of Neuroscience, University of Copenhagen, Denmark*

Abstract: Comprehensive knowledge of the developing human brain is the steppingstone for a fundamental understanding of neurodevelopmental disorders as well as an enlightened view on the path towards each brain cell type. In recent years, cell-precision analysis of human embryonic samples, together with insight from in vitro models such as the self-organizing organoid systems, have uncovered a wealth of information underlying the variety of cell types emerging during human neurodevelopment. We have previously showed that, using a microfluidic controlled in vitro system, termed MiSTR, we could impose an embryologically-inspired rostro-caudal gradient during the neural differentiation of hESCs. This system allowed the consistent recapitulation the neural tube patterning along the neuroaxis, from forebrain to hindbrain, complete with the emergence of a clear midbrain-hindbrain boundary (MHB) signature. Using the MiSTR system, here we investigate the impact of the overexpression of a gene relevant for ventral Midbrain development, LMX1A, in the overall patterning of the neural tube. Unexpectedly, we observed a ventral-to-dorsal shift along the entire rostro-caudal axis despite the presence of ventral cues. We further characterize the formation of the MHB in the MiSTR tissue through single mRNA spatial detection and investigate MHB induction using in an embryoid body system. Finally, we explore the introduction of a second dorso-ventral signaling gradient onto the MiSTR rostro-caudal axis. Overall, we show that the MiSTR system can be used as an important tool to examine and explore the earliest patterning events of the developing human brain, which underlie the organization and origin of the numerous cell types in the embryonic brain.

Keywords: Human embryonic stem cells, early neural patterning, microfluidics

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CHROMOSOMAL BREAKAGES IN EARLY MAMMALIAN EMBRYO DEVELOPMENT IS LINKED TO DNA REPLICATION DYNAMICS

Xu, Shuangyi - *Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University Medical Center, New York, NY, USA*
Palmerola, Katherine - *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Columbia University, New York, NY, USA*
Amrane, Selma - *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Columbia University, New York, NY, USA*
De Los Angeles, Alejandro - *Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA*
Wang, Ning - *Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA*
Baslan, Timour - *Cold Spring Harbor Laboratory, New York, NY, USA*
Egli, Dieter - *Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA*

Abstract: Human development is highly inefficient, with only about one in four oocytes resulting in a viable pregnancy. Chromosomal aneuploidies during mitosis are frequently acquired in mammalian cleavage stage embryos are believed to be an important factor. The genome of the early human embryo is highly unstable, which may result in both lethal, as well as in viable, but disease-causing chromosomal alterations. Previous studies showed that spontaneous and Cas9 induced double-strand breaks (DSBs) often result in chromosome aneuploidies. However, how chromosomal breakages form spontaneously in human embryos and how they cause aneuploidies is not well understood. Here, we show that mammalian embryos show the hallmarks of DNA replication stress, including slow replication fork progression, active replication forks in G2 phase, DNA breakage, incomplete replication in mitosis, DSBs and the conversion of DNA breaks to aneuploidies and chromosomal breakage. By leveraging the single-cell whole-genome sequencing, we use copy number and replication timing analysis to show spontaneous chromosome breaks are mapped to gene-poor and origin-poor regions that complete replication late in S phase. In contrast to human embryos, mouse embryos develop efficiently and spontaneous chromosomal aneuploidies are uncommon. Incubation of mouse embryos with the DNA polymerase inhibitor aphidicolin induces replication-associated chromosomal breakages results in break sites mapped to late replicating regions with low gene and origin density. The induction of DNA replication stress in mouse embryos mimics spontaneous defects in human embryos, including aneuploidies, chromosome breakage and compromised development. Differences in gene expression point to genes involved in DNA replication fork stability and DSB repair as critical determinants of these differences between mouse and human embryo development.

Keywords: human preimplantation embryo, double strand break, chromosome fragility, replication stress, stalled replication fork

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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MODELING AMYLOID BETA PEPTIDE INDUCED ENDOTHELIAL ACTIVATION IN ENGINEERED 3D HUMAN BRAIN MICROVESSELS

Shin, Yu Jung - *Department of Bioengineering, University of Washington, Seattle, WA, USA*
Evitts, Kira - *Department of Bioengineering, University of Washington, Seattle, WA, USA*
Jin, Solhee - *Department of Bioengineering, University of Washington, Seattle, WA, USA*
Howard, Caitlin - *Department of Bioengineering, University of Washington, Seattle, WA, USA*
Young, Jessica - *Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA, USA*
Zheng, Ying - *Department of Bioengineering, University of Washington, Seattle, WA, USA*

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder accompanied by the secretion and deposition of amyloid-beta peptides (Aβ) in the brain. Recent studies highlight a role of vascular dysfunction in the development and progression of AD. However, whether and how Aβ induces endothelial dysfunctions is largely unexplored. Here, we differentiated human induced pluripotent stem cells (hiPSCs), harboring the amyloid precursor protein (APP) Swedish mutation KM670/671NL (Swe22) known to cause familial AD (FAD), into cortical neurons (hiPSC-CNs). We collected conditioned media (CM) from Swe22 hiPSC-CNs and its healthy isogenic control (SweWT) and treated

it on human brain microvascular endothelial cells (HBMECs) in 2D and 3D engineered microvessels to study EC injury in the context of AD. Elisa assay revealed significant elevation of Aβ levels in CM collected from Swe22 hiPSC-CNs compared to CM from SweWT hiPSC-CNs. In 2D, Swe22-CM treated HBMECs showed endothelial activation, characterized by stress fiber formation, and up-regulation of genes associated with leukocyte adhesion (ICAM1, VCAM1, SELE and CCL2). This suggests cytokines and Aβ peptides present in CM drive vascular inflammation and hemodynamic dysfunction. In 3D engineered microvessels, Swe22-CM perfusion led to acute activation of ECs, characterized by the release of von Willebrand factor (VWF) and formation of ultra-large transluminal fibers in the vessel lumen. In contrast, negligible vessel activation was observed in the SweWT CM treated microvessels. To determine if Aβ directly modulates the formation of vWF fibers, we treated BACE1 inhibitors (BACEi) in the hiPSC-CN cultures to inhibit β-secretase and prevent the cleavage of APP into pathogenic Aβ monomers. BACEi treatment decreased production of Aβ in CM and significantly reduced the number of vWF fiber formation within the perfused microvessels. Our findings suggests that the presence of Aβ activates brain ECs, priming for inflammatory and thrombogenic responses within the cerebral microvessels. As vascular inflammation manifests in the early stages of AD neuropathology, the inflammation cycle driven by Aβ may exacerbate AD pathology and augment cognitive impairment.

Funding Source: NIH/NIA R21AG074373 (to YZ and JY)
Keywords: Vascular engineering, Alzheimer's disease, Disease modeling

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IN VITRO MODELING OF ATHEROSCLEROSIS IN HUMAN BLOOD VESSEL ORGANOIDS

Kong, Dasom - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Kim, Nam Gyo - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Lee, Seung-Eun - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Shin, Nari - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Kim, Hee-Yeong - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Kim, Min-Ji - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Choi, Jungju - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*
Kang, Kyung-Sun - *College of Veterinary Medicine, Seoul National University, Seoul, Korea*

Abstract: As modeling of atherosclerosis needs recapitulation of complex interactions with vasculature and immune cells, previous in vitro models have limitation that lack of atherosclerotic phenotypes due to their insufficient vascular structure or cell types. To overcome this, we developed atherosclerotic blood vessel organoids (BVOs) through mimicking the environment by co-culture with monocytes in spinning culture condition. Treatment of enzyme-modified low-density-lipoprotein (eLDL) with TNFα in culture media induced atherosclerosis in BVOs. Since BVO contains multiple cell types including endothelial cells, vascular smooth muscle cells and monocytes/macrophages self-assembled in blood vessel structure, we could observe representative atherosclerotic phenotypes including endothelial dysfunction, inflammatory responses, monocyte accumulation, foam cell formation and fibrous plaque formation in BVOs. Furthermore, we evaluated the inhibitory effects of HMG-CoA reductase inhibitor lovastatin in atherosclerosis BVOs. As the results, lovastatin-treated groups exhibited repressed atherosclerotic phenotypes compared to vehicle-treated atherosclerotic BVOs. These results suggest that atherosclerotic BVO is an advanced in vitro model which is suitable for large scale drug discovery and further elucidation of mechanisms.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A4A4078907).
Keywords: Blood vessel organoids, Atherosclerosis, Inflammatory disease

TOPIC: EPITHELIAL_GUT

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TRANSCRIPTIONAL REGULATION OF CELL STATE TRANSITIONS IN INTESTINAL EPITHELIAL CELLS DURING DEVELOPMENT

Hansen, Stine L. - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Larsen, Hjalte - *reNEW - NNF Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Pikkupeura, Laura - *NNF Center for Basic Metabolic Research, University of Copenhagen, Denmark*
Maciag, Grzegorz - *reNEW - NNF Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Guiu, Jordi - *Cell Plasticity and Regeneration, IDIBELL - Institut d'Investigació Biomèdica de Bellvitge, Barcelona, Spain*
Clement, Ditte - *reNEW - NNF Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Johansen, Jens - *Biotech Research & Innovation Centre, University of Copenhagen, Denmark*
Sandelin, Albin - *Biotech Research & Innovation Centre, University of Copenhagen, Denmark*
Lerdrup, Mads - *Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark*
Jensen, Kim - *reNEW - NNF Center for Stem Cell Medicine, University of Copenhagen, Denmark*

Abstract: Generation of functional tissues during development involves sequential cell state transitions ensuring the progressive maturation of immature fetal progenitor cells. During intestinal development, a population of equipotent fetal progenitors give rise to stem cells which maintain the adult intestinal epithelium through a process of dynamic equilibrium. Despite extensive knowledge of the regulation of adult intestinal stem cell behavior, the molecular regulators driving the transition of cells from a fetal to an adult state remains largely unknown. Here we investigated the transcriptional basis underlying such cell state transitions during intestinal maturation using fetal intestinal organoids. Culturing intestinal epithelial cells from mouse embryos under defined three-dimensional conditions gave rise to cultures of fetal organoids stably maintaining their immature characteristics without spontaneous maturation. scRNA-sequencing revealed the generation of a small number of cells gaining markers of mature epithelial cells during steady-state culture, confirming the potential of fetal cells to transition towards maturation in a process occurring at low frequency in culture. In order to investigate the genetic bias of this transition, we performed a multiparameter

phenotypic CRISPR/Cas9 KO screen in fetal organoids targeting transcriptional modifiers. Through the screen we identified factors that alter the propensity of cell state transition in the intestinal epithelium during development, either by facilitating the transition towards adult-like cells or by further enforcing the fetal-like state. As regeneration of intestinal tissue following injury has been demonstrated to rely on the transient reprogramming of the adult epithelium into a fetal-like state, our findings hold the promise of also elucidating the transcriptional basis of cellular reprogramming in disease, potentially benefitting patients suffering from inflammatory bowel diseases.

Funding Source: The project is funded by ERC and the Novo Nordisk Foundation Center for Stem Cell Medicine reNEW (grant number NNF21CC0073729).
Keywords: Intestinal development, Cell state transitions, Organoids

TOPIC: EPITHELIAL_LUNG

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MODELLING THE EFFECT OF EXTRACELLULAR MATRIX DYSREGULATION IN PULMONARY FIBROSIS USING HIPSC DERIVED-ALVEOLAR ORGANOIDS AND SELF-ASSEMBLING HYDROGELS

Reed, Liam - *Cancer and Stem Cells, University of Nottingham, UK*
Serna-Valverde, Ana Lilia - *Cancer and Stem Cells, University of Nottingham, UK*
Cuevas-Ocana, Sara - *Cancer and Stem Cells, University of Nottingham, UK*
Merry, Cathy - *Cancer and Stem Cells, University of Nottingham, UK*
Hannan, Nicholas - *Cancer and Stem Cells, University of Nottingham, UK*

Abstract: Idiopathic pulmonary fibrosis (IPF) remains a lethal disease and despite recent advancements in clinical care life expectancy is less than 5 years following diagnosis. Repeated damage to the lung epithelium, in particular to Type II alveolar epithelial cells (AECs), the stem cells of the alveoli, is considered the main driver of disease progression and leads to chronic inflammation and fibrosis. While IPF has no known driver mutations, familial IPF is known to be caused by mutations in several important respiratory-associated genes including NKX2.1, surfactant proteins, ABCA3 and MUC5B. Dysregulated extracellular matrix (ECM) remodelling and excessive ECM deposition is central to IPF pathogenesis. However, current models exploring aberrant epithelium-ECM interactions underlying fibrosis progression in IPF have yet to deliver changes in disease prognosis. This is owing to widespread use of animal models of respiratory fibrosis coupled with challenges in maintaining human primary Type II AECs in vitro and reliance on undefined basement membrane complexes such as Matrigel™ for fibrosis modelling. Robust stem cell platforms for generating Type II AECs that can be incorporated into ECM formulations that mimic the ECM environment of healthy and fibrotic lung tissue are needed for more accurate respiratory fibrosis modelling. To address this, we have optimised a differentiation platform capable of generating Type II AECs from hiPSCs with a pulmonary fibrosis genetic background. Using a homogenous population of Type II AECs, we model the effect of progressive fibrosis on cellular phenotype using a self-assembling peptide hydrogel. These hydrogels are well defined, biocompatible, and they form ECM-like matrices that can be tuned to mimic the microenvironment of diseased tissues of interest. Importantly, they allow us to generate versatile combinations and ratios of ECM components and control biological properties of the ECM such as stiffness. Using whole genome spatial genomics, our platform allows us to model early and late-stage respiratory fibrosis and reveals mechanisms of how biological properties and composition of the ECM impacts gene expression and function of Type II AECs, and its contribution to IPF disease progression.

Funding Source: The Masonic Charitable Foundation British Lung Foundation Medical Research Council
Keywords: hiPSC-derived organoids, Idiopathic Pulmonary Fibrosis, Extracellular Matrix

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GENERATION OF HUMAN NASAL AIRWAY EPITHELIAL CELLS FROM INDUCED PLURIPOTENT STEM CELLS

Li, Yingchun - *Center for Genes, Environment and Health, National Jewish Health, Denver, CO, USA*
Goldfarbmuren, Katherine - *Center for Genes, Environment and Health, National Jewish Health, Denver, CO, USA*
Seibold, Max - *Center for Genes, Environment and Health, National Jewish Health, Denver, CO, USA*

Abstract: Primary human nasal stem cells can easily be isolated and cultured from human subjects allowing personalized cellular models of airway epithelium function. However, cells isolated directly from patients carry programming that influences their cellular/molecular behavior, creating biases in results generated from these primary cells. The generation of induced pluripotent stem cells (iPSCs) from individual donors and then derivation of nasal basal stem cells from these iPSCs, would produce a potentially unlimited source of “scar-free” basal stem cells, with both research modeling and regenerative medicine applications. Until now, it is unclear how to generate nasal airway epithelial cells from pluripotent stem cells, a process that will be critical for understanding nasal airway development, modelling upper respiratory disorders, and improving nose specific cell-based therapies. Here we reprogrammed donor derived airway basal stem cells to iPSCs with a virus-, DNA-, and integration-free RNA-based method. Through stage specific stepwise differentiation protocol, we successfully differentiated donor derived iPSCs to non-neuroectoderm, cranial placode ectoderm, and then to nasal airway progenitors. We further directed nasal airway progenitors to nasal epithelial organoids via 3D culture methods. Pure nasal basal stem cells were further specified and procured, without the aid of genetic manipulation, through dual SMAD pathway inhibition. The induced nasal basal cells (iNBCs) generated by this method were positive for basal stem cell markers KRT5, TP63, and Ki67. Interestingly, lineage specific gene expression assay, we identified these iNBCs highly expressed ectoderm-derived nose specific genes (TFAP2A and PAX6), but not endoderm-derived lung specific gene (NKX2.1). Importantly, these iNBCs can be differentiated into a highly consistent pseudostratified epithelium (basal cells, ciliated cells, mucus cells, and club cells) via standard air-liquid interface differentiation. In summary, we have developed a novel method to reliably generate a population of human nasal airway basal stem cells capable of producing normal mucociliary epithelium. Our methods represent a significant advance in the

quest for regenerative therapeutic approaches applied to the human nasal airway epithelium.

Keywords: iPSCs, Nasal airway epithelial cells, cranial placode ectoderm

TOPIC: EPITHELIAL_SKIN

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DEFINING THE ROLE OF GLUTAMINOLYSIS IN HAIR FOLLICLE STEM CELL INDUCED CUTANEOUS SQUAMOUS CELL CARCINOMAS

Cerrillos, Victoria - *MCDB, University of California, Los Angeles, Granada Hills, CA, USA*

Abstract: Cutaneous squamous cell carcinoma (cSCCs) are most commonly caused by chronic UV radiation on the head, neck and limbs. cSCCs exhibit the Warburg effect, in which cancer cells rewire their metabolism to favor high glucose uptake and lactate production to sustain growth, metastasis, and immune suppression. Lactate dehydrogenase (Ldha) is the main enzyme used for lactate overproduction, but recent studies suggest that SCC growth is also dependent on glutaminolysis. During this process, cancer cells use the amino acid glutamine to sustain themselves. Glutaminase (Gls) is the first enzyme that catalyzes glutaminolysis and has been found to be overexpressed across a variety of cancers. Hair follicle stem cells (HFSCs) have been found to be the cells of origin for cSCCs, but targeting glutaminolysis during cSCC initiation has not been studied. We hypothesize that the genetic deletion of Gls or a combination of Gls and Ldha in the cells of origins for cSCCs will target glutaminolysis and lactate overproduction, thus preventing cSCC tumorigenesis. Using an in vivo model has allowed us to understand the transformation of HFSCs into cancerous cells. Moreover, utilizing a two-stage skin chemical carcinogenesis model has elucidated the skin to tumor metabolic evolution and the effectiveness of glutaminolysis regulation of normal physiology and cancer.

Keywords: Cancer, Metabolism, Tumorigenesis

TOPIC: EYE AND RETINA

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MODELLING EARLY RETINAL DEVELOPMENT IN RETINAL ORGANOIDS DERIVED FROM RETINITIS PIGMENTOSA TYPE 11 WITH PRPF31 MUTATIONS

Schjølberg, Jørn-Ove - *Department of Clinical and Molecular Medicine (IKOM), Norwegian University of Science and Technology, Trondheim, Norway*
Bjørås, Magnar - *Department of Clinical and Molecular Medicine (IKOM), NTNU/OUH, Trondheim, Norway*
Wang, Wei - *Department of Clinical and Molecular Medicine (IKOM), NTNU, Trondheim, Norway*

Abstract: Retinitis pigmentosa type 11 (RP11) is a hereditary retinal disease due to mutations in one copy of the Pre-mRNA processing factor 31 (PRPF31) gene. RP11 generally debuts in early adulthood with impaired night vision, narrowing of the visual field, and in the final stage, loss of central vision. Genetic defects in the PRPF31 gene leads to progressive cell death in the retinal layers, primarily affecting the retinal pigment epithelial layer and secondarily in the photoreceptor layer. iPSC derived- human organoids are robust models that can reveal new mechanistic insight into the pathology of diseases and can be used as experimental platforms for drug screening and gene therapy. Here, patient-derived skin fibroblasts were reprogrammed into iPSC and further differentiated into early retinal organoids to investigate if the PRPF31 mutation affects the early stages of retinal development. Characterization of retinal markers were examined from the iPSC stage until the emergence of photoreceptor progenitors. The PRPF31 expression in patient-derived iPSC and early retinal organoids measured by qPCR was significantly lower than in healthy control samples. However, preliminary data from immunostaining suggest that retinal markers were not significantly altered in the early retinal organoid stages, and they successfully developed photoreceptor precursors. In addition, the morphology of optic vesicles was not particularly affected. Ongoing studies of transcriptome, proteome and epigenome will be presented.

Keywords: Retinal organoid, hiPSC, RP11 - PRPF31

TOPIC: HEMATOPOIETIC SYSTEM

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OPTIMIZING DERIVATION OF DECIDUAL-TYPE NATURAL KILLER (DNK) CELLS FROM HUMAN PLURIPOTENT STEM CELLS

DaCosta, Carly - *Biology, San Diego State University, San Diego, CA, USA*
Chu Cheung, Virginia - *Pathology, University of California San Diego, CA, USA*
Fierro, Manuel - *Regenerative Medicine, University of California San Diego, CA, USA*
Caron, Christine - *Pathology, University of California San Diego, CA, USA*
Bui, Jack - *Pathology, University of California San Diego, CA, USA*
Kaufman, Dan - *Regenerative Medicine, University of California San Diego, CA, USA*
Horii, Mariko - *Pathology, University of California San Diego, CA, USA*
Parast, Mana - *Pathology, University of California San Diego, CA, USA*

Abstract: Decidual natural killer (dNK) cells are immune cells that provide a well-balanced microenvironment to enable proper development and functioning of the placenta. Abnormal frequencies of dNK are found in cases of preterm birth compared to term normal pregnancy; however, the impact of dNK in preterm birth is unknown due to the lack of a modeling system. Our research aims to establish a modeling system using human pluripotent stem cells (hPSC) to study dNK and their role in preterm birth. A previously published natural killer (NK) cell differentiation protocol has used both human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) over several defined stages: hematopoietic stem-progenitor cell (HSPC)-specified embryoid body (EB) development, NK cell differentiation, and NK cell expansion. We differentiated WA09/H9 hESCs to HSPCs using the spin EB method and cultured in HSPC induction media containing stem cell factor, bone morphogenetic protein 4, vascular endothelial growth factor, and rho kinase inhibitor. Subsequently, the EBs were replated for NK cell differentiation in NK Differentiation Media as previously published for additional 28 days. Differentiation was assessed by flow cytometry at each step. For comparison, we characterized NK cells from term placental basal plate (decidua) and peripheral blood and found that unlike in blood, the major population of NK

cells in the placenta was CD56+/CD16-. We were able to replicate HSPC induction with >10% CD34+ cells at day 6 of differentiation. Twenty-eight days after replating the EBs for NK cell differentiation, ~ 96% of the cells were CD45+ immune cells with ~65% composed of CD56+/CD16- NK cells, similar to term dNK cells. Following expansion, however, these cells gain CD16 expression, more similar to peripheral blood NK phenotype. In conclusion, we are able to generate decidual-like NK cells CD45+CD56+CD16- at the end of differentiation. We are currently working on optimizing the protocol to increase differentiation efficiency, establish conditions for expansion of the cells without gain of CD16, and compare primary and hPSC-derived dNK through RNA sequencing. This would bring us closer to establishing a coculture system with hPSC-derived dNK and trophoblasts (placental epithelial cells), in order to model the maternal-fetal interface.

Funding Source: Modeling human trophoblast-NK cell interactions in term and preterm birth: R01-HD-102639
Keywords: decidual natural killer cells, placenta, differentiation

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ASSESSING HEMATOPOIETIC STEM CELL FITNESS WITHIN A NANOBIOREACTOR IN MICROGRAVITY

Pham, Jessica -
Oliver, Isabelle - *Division of Regenerative Medicine, Department of Medicine, University of California, San Diego, La Jolla, CA, USA*
Balaian, Larisa - *Division of Regenerative Medicine, Department of Medicine, University of California, San Diego, La Jolla, CA, USA*
Jamieson, Catriona - *Division of Regenerative Medicine, Department of Medicine, University of California, San Diego, La Jolla, CA, USA*

Abstract: Microgravity coupled with high radiation exposure aboard the ISS provides a unique environment to simulate and study response to injury, inflammatory signaling, aging, and (pre-) malignant transformation of normal human hematopoietic stem cells (HSCs) in an accelerated timeframe. The NASA Twins study suggests that genomic, epigenomic, epitranscriptomic, and proteomic changes may detrimentally impact hematopoietic stem and immune cell fitness and induce stem cell exhaustion. Moreover, changes indicative of pre-cancer stem cell generation such as increased chromosome translocations and inversions occurred and persisted post-flight. Additionally, a recent publication entitled Multisystem Toxicity in Cancer: Lessons from NASA's Countermeasures Program found significant similarities between the multisystem physiological toxicities in cancer patients and during spaceflight. These reports highlight the benefits of studying injury response, changes in mutational profiles and cancer evolution in microgravity at the stem cell level. For this study, we designed a novel bioreactor system to support the culture of donor-derived human HSCs in low Earth orbit (LEO). A sponge matrix and stromal cells model the microenvironment HSCs reside in within the bone marrow niche. Testing on Earth confirmed our system's ability to maintain stem cell fitness over several weeks. To assess stem cell physiology in LEO over time, we lentivirally transduce a reporter into the HSCs pre-flight, which allows for cell cycle tracking via fluorescence imaging. This will provide data for assessment of stem cell health, maintenance and functionality. Furthermore, we will be analyzing mutational status post-flight, with a focus on signatures we have previously connected to (pre-) malignant transformation via RNA sequencing analysis. Our bioreactors launched onto the ISS as part of the SpaceX CRS-24 mission on Dec 21, 2021. This investigation may provide valuable insights into the maintenance of hematopoietic stem cell health and functionality, response to injury through accumulation of mutations and, eventually, the mechanisms fueling long-term (pre-) malignant transformation into leukemia stem cells.

Keywords: Hematopoietic stem cell maintenance, Leukemia stem cell generation, hematopoietic stem cell niche model

TOPIC: IMMUNE SYSTEM

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GLUCOCORTICOID PRETREATMENT OF BONE MARROW CELLS PRIOR TO ALLOGENEIC TRANSPLANTATION IN MICE REDUCES GRAFT-VERSUS-HOST DISEASE

Ayala, Luis A. - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*
Varady, Erika - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*
Nguyen, Pauline - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*
Scarfone, Vanessa - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*
Zhou, Cuiwen - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*
Inlay, Matthew - *Molecular Biology & Biochemistry, University of California, Irvine, CA, USA*

Abstract: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potential curative therapy for many blood disorders. However, it remains underutilized due to complications that can arise following allo-HSCT, namely graft failure and Graft-versus-Host disease (GvHD). In acute GvHD, donor immune cells become activated and attack the patient's tissues. Currently, the most common initial treatments are powerful immunosuppressives, like glucocorticoids (GCs) which aim to reduce the severity of GvHD and prevent death. Despite the optimized GC-regimen, many patients succumb to GvHD-related death, thus highlighting a need for novel strategies which make allo-HSCT a safer treatment option. Previous research demonstrated that GC-pretreatment of human cord blood stem cells enhanced their engraftment upon transplantation into mice, however, they did not assess their impact on GvHD. Since GCs are immunosuppressive, we reasoned that treating BM and spleen cells would reduce the reactivity of T cells to being transplanted in an allo-HSCT. In this study, we hypothesized that pretreatment of donor cells prior to transplantation would reduce GvHD severity and incidence in mice that receive an allo-HSCT. Strikingly, we observed that GC treatment killed T cells in vitro but were able to repopulate following transplantation and appeared less alloreactive. We observed reduced GvHD in mice that received GC treated cells when compared to mice that received vehicle treated cells. Moreover, GC treatment induced apoptosis of conventional T cells while sparing regulatory T cells, suggesting a potential mechanism by which GvHD is reduced in our model. Our results implicate an important role for regulatory T cells in maintaining allogeneic tolerance and highlighting a potential strategy which would make allo-HSCT a safer therapeutic.

Funding Source: EDUC4-12822 R01AG055524
Keywords: Bone Marrow Transplantation, Graft-versus-Host Disease, Regulatory T cells

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CRISPR-CAS9 WHOLE GENOME SCREENS IDENTIFY MECHANISMS FOR IMMUNOTHERAPEUTIC VERSUS CHEMOTHERAPEUTIC ANTHRACYCLINE TREATMENT

Perry, John M. - *Pediatrics, Children's Mercy Kansas City, MO, USA*
Nemechek, Jacqelyn - *Pediatrics, Children's Mercy Kansas City, MO, USA*
Stockard, Bradley - *Pediatrics, Children's Mercy Kansas City, MO, USA*
Schroeder, Kealan - *Pediatrics, Children's Mercy Kansas City, MO, USA*
Pace, Jennifer - *Pediatrics, Children's Mercy Kansas City, MO, USA*

Abstract: Cancer develops only in the context of a failing immune system. Normally, an immunosurveillance system identifies and eliminates potentially tumorigenic cells. For cancer to progress, tumorigenic cells must evade or undermine this system. Expression of immune checkpoints is a major mechanism underlying immune escape. Leukemia stem cells (LSCs), which are enriched in tumorigenic activity and are particularly resistant to anticancer therapies, express multiple immune checkpoints to evade cytotoxic T cells. However, we recently showed that the long-used chemotherapy agent doxorubicin can be repurposed as a targeted therapy to inhibit immune checkpoint expression and expose LSCs to cytotoxic T cell-mediated elimination. This effect only occurs well below the dose typically used in the clinic. Here, we use CRISPR-Cas9 whole genome screens to investigate the effects of differential doses of doxorubicin on leukemia cells. Interestingly, while low, non-lethal exposures to doxorubicin have immunotherapeutic effects by repressing immune checkpoint expression, intermediate doses initiate immunogenic cell stress and death by inducing cell surface expression of calreticulin. These low to intermediate dose responses are further distinguished from high, chemotherapy doses by the induction of non-immunogenic necrotic cell death of most cancer cells with induction of resistance mechanisms in rare, surviving cells. Overall, our work indicates that current use of a common chemotherapy agent may be undermining immunotherapy options for cancer patients but also suggests how it might be used to enhance these options.

Funding Source: Research reported in this publication was supported by Children's Mercy Kansas City, Braden's Hope for Childhood Cancer, Noah's Bandage Project, and the V Foundation.
Keywords: Oncoimmunology, Immune Checkpoint, CRISPR-Cas9

TOPIC: KIDNEY

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GENERATING ROBUST, SCALABLE PROTOCOLS FOR THE DIFFERENTIATION OF KIDNEY ORGANOIDS AND PODOCYTES FROM HIPSCS WITH FUNCTIONAL READOUTS FOR SCREENING DRUG CANDIDATES TARGETING A RANGE OF RENAL DISEASES

Morse, Brian P. - *Velocigene, Regeneron Pharmaceuticals Inc., Tarrytown, NY, USA*
Chiao, Eric - *Velocigene, Regeneron, Tarrytown, NY, USA*
Devalaraja, Kishor - *Cardiovascular & Renal Research, Regeneron, Tarrytown, NY, USA*
Gagliardi, Anthony - *Velocigene, Regeneron, Tarrytown, NY, USA*
Halasz, Gabor - *Molecular Profiling and Data Science, Regeneron, Tarrytown, NY, USA*
Kerk, John - *Velocigene, Regeneron, Tarrytown, NY, USA*
Zhu, Yuan - *Molecular Profiling and Data Science, Regeneron, Tarrytown, NY, USA*

Abstract: Several protocols have been published to optimize the differentiation of hiPSCs toward human kidney organoids. Most of these methods use formats not well-suited to large-scale screening or require substantial technical optimization for various cell lines. Using a combination of these published protocols, we have developed a robust, reproducible, and scalable protocol to generate kidney organoids with functional properties similar to the human adult kidney. Through RNA sequencing and immunofluorescence, we have shown that our kidney organoids contain cell populations key to the structure of the nephron, including the glomerulus, the proximal tubule, and the distal tubule. A notable difference between our protocol and those found in the literature is that ours appears to produce proportionally more podocyte-like cells. Functionally, the various cell types present in our kidney organoids respond to molecules associated with kidney damage or disease. Cisplatin, a chemotherapeutic that affects the cells of the proximal tubule of the nephron, induces the secretion of kidney injury molecules. Another molecule that has been linked to various kidney diseases is the cytokine interferon gamma, which causes an increase in APOL1 expression in podocytes. In addition to organoids, we also developed a complementary approach by differentiating podocytes from iPSCs into a microfluidics-based, glomerular filtration model. Using this model, we were able to establish a selectively permeable slit diaphragm of our wild type cells and a knock-out of our protein of interest. We were also able to dose the microfluidics chips with interferon gamma and observe proteinuria, or the breakdown of the slit diaphragm at different concentrations. Combining our scalable protocols with the ability to genetically engineer genetic variants via CRISPR/Cas9, this platform could be used to screen for possible drug candidates targeting a range of renal disease variants.

Funding Source: Regeneron
Keywords: Kidney, Organoid, Microfluidics

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A PROTOCOL FOR THE DIRECTED DIFFERENTIATION OF ADRENOCORTICAL PROGENITOR CELLS

McAlpine, Jessica - *Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA*
Zeltner, Nadja - *Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA*

Abstract: The adrenal gland is a vital endocrine organ that secretes hormones and catecholamines in response to internal and external changes. It is composed of two developmentally and functionally distinct regions, arranged into a core and outer shell. The core, or medulla, arises from the neural crest and mediates the short-term stress response via catecholamine release to elicit a "fight-or-flight" response. The cortex, which has a mesodermal origin, regulates homeostasis, metabolism, and sex development as well as mediating long term stress responses through the release of a variety of hormones. The developing cortex is marked by a strong upregulation of the steroidogenic transcription factor NR5A1 in a subset of intermediate mesoderm (IM)-derived cells. Disruption of adrenocortical function has dire physiological and developmental consequences. Animal models of adrenocortical disease and dysfunction have been supportive but often don't recapitulate human adrenal physiology due to differences in hormone production. A human pluripotent stem cell (hPSC)-derived platform for studying human adrenocortical development and disease offers the opportunity to perform research in a more physiologically relevant context, isolate specific cell types of interest, and study human development. However, to date, no directed differentiation protocol for generating adrenocortical cells has been published. Here, we describe a protocol for directed differentiation of adrenocortical progenitors from hPSCs by modulating BMP, FGF, PKA and Wnt signaling. By RT-qPCR we observe upregulated expression of coleomic epithelium genes WT1 and GATA4 followed by strong upregulation of steroidogenesis genes NR5A1, STAR, and NR0B1 without significant upregulation of markers such as SOX9 or PAX2 that indicate a gonad or kidney cell identity respectively. This model may allow researchers to investigate human adrenal development, function, and interaction with other cell types in vitro as well as bring us one step towards cell replacement therapy for adrenal gland disorders.

Keywords: Adrenal Gland, Protocol, Directed Differentiation

TOPIC: MUSCULOSKELETAL

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SCALABLE HUMAN SKELETAL MYOCYTES BY OPTI-OX REPROGRAMMING OF IPSCS FOR THE STUDY OF MUSCLE BIOLOGY AND DISEASE MODELLING

Bernard, William G. - *Cell Type Development, bit.bio, Cambridge*

Abstract: 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 such as muscular dystrophies and metabolic disorders. 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 and complex differentiation protocols. We have developed an optimised inducible system (opti-ox) that enables tightly controlled expression of transcription factors (TF) improving cellular reprogramming approaches for the differentiation of hiPSCs. TF induction leads to shutdown of the core pluripotency network and activation of key myogenic factors including myosin heavy chain. Cryopreserved ioSkeletal Myocytes homogeneously express the key proteins of the myofilaments Desmin, Dystrophin and Titin, and form striated and multinucleated myocytes that contract in response to acetylcholine by 10 days post-revival. The skeletal muscle phenotype and culture homogeneity have been further analysed by RNA sequencing to provide in depth characterization. Importantly, ioSkeletal Myocytes produce a highly pure Myosin Heavy Chain positive population of cells within only 4 days of thawing and are amenable to high-throughput screening pipelines. In addition, we employed CRISPR/Cas-9 gene editing to introduce deletions in the DMD gene of the wild type ioSkeletal Myocytes to mimic Duchenne Muscular Dystrophy (DMD). This isogenic system will improve screen specificity and accelerate drug development for DMD.

Funding Source: N/A
Keywords: Skeletal Myocytes, Duchenne Muscular Dystrophy, Disease Modelling

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HUMAN PLURIPOTENT STEM CELL-DERIVED CRANIAL AND SPINAL MOTOR NEURONS REVEAL TRANSCRIPTIONAL MECHANISMS BETWEEN RESISTANT AND SENSITIVE MOTOR NEURONS IN SPINAL MUSCULAR ATROPHY

Martinat, Cécile - *I-STEM, INSERM, Corbeil Essonnes, France*
Januel, Camille - *I-STEM, INSERM UMR861, Corbeil-Essonnes, France*
Jarrige, Margot - *I-STEM, I-STEM, Corbeil-Essonnes, France*
Leteur, Céline - *I-STEM, Evry, France*
Polentes, Jerome - *I-STEM, Evry, France*
Polvèche, Hélène - *I-STEM, I-STEM, Corbeil-Essonnes, France*

Abstract: Spinal muscular atrophy is an autosomal recessive disease caused by the Somatic motor neurons are selectively vulnerable in spinal muscular atrophy (SMA), which is caused by a deficiency of the ubiquitously expressed survival of motor neuron protein. While spinal motoneurons are highly vulnerable, some motor neuron groups, including cranial motoneurons, which innervate the face and eye muscles, are spared. Understanding the mechanisms underlying this selective vulnerability is crucial to determine how the loss of a ubiquitously expressed protein can induce degeneration in a select cell type and consequently to determine new therapeutic pathways. However, progresses towards identification of such mechanisms have been hampered by the restricted access to these cellular types in humans. We took advantage of human pluripotent stem cells (hiPSC) to generate homogenous populations of spinal and cranial motoneurons. Both populations of motoneurons displayed a similar decreased SMN expression. However, SMA hiPSC-derived cranial motoneurons were found to be spared whereas SMA hiPSC-derived spinal motoneurons exhibited a defective connectivity to their muscular targets as well as a decreased suvival. Comparative transcriptional analysis between resistant and vulnerable SMA motoneurons revealed the potential contribution of genes involved in neuro-

transmission and neurite outgrowth that may aid in the maintenance of a functional neuromuscular synapse and thus contribute to the selective resistance of certain motor neurons in SMA Altogether, our results described the possibility to capture in vitro the selective vulnerability associated with SMA and should facilitate the identification of new therapeutic targets toward preserving vulnerable motoneurons.

Funding Source: Laboratoire d'Excellence Revive (ANR-10-LABX-73), the doctoral school « Innovation Thérapeutique, du fondamental à l'appliqué » (ED 569) from Paris Saclay University, Agence Nationale de la Recherche (ANR)
Keywords: disease modeling, neuromuscular disease, motoneurons

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A SPATIALLY PATTERNED IN VITRO SOMITOGENESIS MODEL USING MICROFLUIDICS

Liu, Yue - *Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*
Fu, Jianping - *Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*

Abstract: Somites, the precursors of various important structures such as skeleton muscle and vertebrae, lay a foundation for vertebrate body plan during the early embryo development. During its derivation from presomitic mesoderm (PSM), somite exhibits a distinctive segmented morphology that is usually connected to the periodic gene expression in PSM, the segmentation clock. However, there is still a lack of a reliable human-specific model that enables both in-depth mechanistic investigation and applications such as disease research. In this study, we utilized human embryonic stem cells and microfluidic devices to generate a somite model that recapitulates the anterior-posterior patterning. Combining immunostaining and transcriptomic analysis, we confirmed the cellular identities of somite and PSM. Spatial and temporal characterizations of somite structure and segmentation clock are performed to investigate the somitogenesis dynamics.

Keywords: somitogenesis, microfluidics, patterning

TOPIC: NEURAL

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THREE-DIMENSIONAL DIRECT REPROGRAMMING APPROACH TO MODEL HUMAN GLIA CONVERSION INTO NEURONS

Giacomoni, Jessica - *Experimental Medical Science, Lund University, Lund, Sweden*
Bruzelius, Andreas - *Experimental Medical Science, Lund University, Lund, Sweden*
Habekost, Mette - *Experimental Medical Science, Lund University, Lund, Sweden*
Rylander Ottosson, Daniella - *Experimental Medical Science, Lund University, Lund, Sweden*
Storm, Petter - *Experimental Medical Science, Lund University, Lund, Sweden*
Fiorenzano, Alessandro - *Experimental Medical Science, Lund University, Lund, Sweden*
Parmar, Malin - *Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Parkinson's disease (PD), one of the most common neurodegenerative disorders, is primarily characterised by progressive loss of dopamine (DA) neurons in the ventral midbrain. The relatively focal degeneration in PD makes it a good candidate for cell replacement therapies, and efforts are on their way to use stem cell derived-DA neurons in clinical trials. An emerging alternative approach to cell transplantation is in vivo reprogramming, where resident glia is converted into neurons directly inside the brain. Proof-of-concept that in vivo conversion can be a viable option has been provided in rodent studies but relevant pre-clinical models where human cells are converted are lacking. We have previously established a renewable and reproducible stem cell-based system of human glial progenitor cells for direct neural conversion and identified optimal combinations of fate determinants for the generation of functional DA neurons in vitro. We have now developed a 3D culture model for direct conversion with the idea that better recapitulation of the complexity of a 3D space more closely mimics conversion in vivo. We show that cells converted in the 3D system develop into functionally mature DA neurons at a higher efficiency and faster pace than neurons converted in 2D. We use single-nucleus transcriptomics to map glia heterogeneity, explore neuronal lineage diversity after direct cell conversion and to further understand the reprogramming competence of different subtypes of glial progenitors. Our data show that reprogramming in 3D increases conversion efficiency, accelerates the reprogramming process and generates mature and functional DA neurons within 2 weeks, making it a valuable model for direct conversion of human glia.

Funding Source: Swedish Research Council; Knut and Alice Wallenberg Foundation
Keywords: Neuronal reprogramming, Spheroids, hOPCs

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THE NEUROPROTECTIVE EFFECTS OF EXOSOMES DERIVED FROM TSG101-OVEREXPRESSING HUMAN NEURAL STEM CELLS IN AN IN VITRO STROKE MODEL AND AN IN VIVO MIDDLE CEREBRAL ARTERY OCCLUSION MODEL

Choi, Yunseo - *Department of Biology Education, Korea National University of Education, Chungju-si*
Yoon, Eun-jung - *Department of Counseling, Health, and Kinesiology, College of Education and Human Development Texas A&M University-San Antonio, Texas, TX, USA*
Kim, Tae Myoung - *Central Research Institute, Designed Cells Co., Cheongju, Korea*
Choi, Ehn-Kyoung - *Central Research Institute, Designed Cells Co., Cheongju, Korea*
Kim, Yun-Bae - *Central Research Institute, Designed Cells Co., Cheongju, Korea*
Park, Dongsun - *Department of Biology Education, Korea National University of Education, Cheongju, Korea*

Abstract: Although tissue-type plasminogen activator was approved by the FDA for early reperfusion of occluded vessels, there is a need for an effective neuroprotective drug for stroke patients. In this study, we established tumor susceptibility gene (TSG)101-overexpressing human neural stem cells (F3.TSG) and investigated whether they show enhanced secretion of exosomes and whether treatment with exosomes during reperfusion alleviates ischemia-reperfusion-mediated brain damage. F3.TSG cells secreted higher amounts of exosomes than the parental F3 cells. In N2A cells subjected to oxygen-glucose deprivation (OGD), treatment with exosomes or co-culture with F3.TSG cells

significantly attenuated lactate dehydrogenase release, the mRNA expression of pro-inflammatory factors, and the protein expression of DNA damage-related proteins. In a middle cerebral artery occlusion (MCAO) rat model, treatment with exosomes, F3 cells, or F3.TSG cells after 2 h of occlusion followed by reperfusion reduced the infarction volume and suppressed inflammatory cytokines, DNA damage-related proteins, and glial fibrillary acidic protein, and upregulated several neurotrophic factors. Thus, TSG101-overexpressing neural stem cells showed enhanced exosome secretion; exosome treatment protected against MCAO-induced brain damage via anti-inflammatory activities, DNA damage pathway inhibition, and growth/trophic factor induction. Therefore, exosomes and F3.TSG cells can affect neuroprotection and functional recovery in acute stroke patients.

Keywords: exosome, neural stem cells, Neuroprotection

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STIMULATOR OF INTERFERON GENES (STING) PATHWAY ACTIVATION IN HUMAN IPSC-DERIVED NEURONS MODELING AMYOTROPHIC LATERAL SCLEROSIS

Marques, Christine - *Neurology, Massachussets General Hospital, Charlestown, MA, USA*
Dorfman, Katherine - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Sung, Joon - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Song, Catherine - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Adler, Michelle - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Kavuturu, Amey - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Held, Aaron - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Aguilar, Corey - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Petrucelli, Leonard - *Neuroscience, Mayo Clinic, Jacksonville, FL, USA*
Oakley, Dereck - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Robey, Ian - *Southern Arizona VA Healthcare System, VA Biorepository Brain Bank, Tucson, AZ, USA*
Hyman, Bradley - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Lagier-Tourenne, Clotilde - *Neurology, Massachusetts General Hospital, Boston, MA, USA*
Wainger, Brian - *Neurology, Massachusetts General Hospital, Boston, MA, USA*

Abstract: The type-I interferon (IFN) response initiated by the stimulator of interferon genes (STING) is an established innate immune response to pathogens but also has been implicated in neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), a rapidly progressing and fatal disorder of the motor nervous system. While these studies have focused on immune cells themselves, cell-autonomous activation of innate immune signaling pathways within neurons can also occur and may play roles in neurodegenerative diseases. However, the role of STING signaling in driving this neuronal response remains unknown and represents the aim of the study. We hypothesize that STING signaling dysregulation within neurons contributes to their degeneration in ALS. We first performed an unbiased analysis of published RNA-seq datasets of ALS spinal motor neurons and found consistent upregulation of several components of the STING pathway and IFN-1 genes. Using human iPSC lines from a range of familial ALS patients as well as mutations introduced by gene editing to create isogenic disease and control pairs, we showed a consistent increase in STING activation within ALS iPSC-derived spinal motor and cortical neurons compared to control. Activation of STING increased IFN-1 markers and decreased neuronal survival, whereas blocking STING decreased IFN-1 and improved neuronal. We validated these findings in vivo, observing increased STING activation selectively in vulnerable layer V neurons from an adeno-associated virus (AAV) C9orf72 model mice compared to control mice. Finally, we confirmed the relevance of these findings by demonstrating similar increased STING protein in layer 5 cortical pyramidal neurons from ALS postmortem brains (n=13) compared to either Alzheimer's (n=8) or non-neurological (n=8) control ones. Thus, our study demonstrates STING pathway activation in ALS neurons, with complementary findings among iPSC, mouse, and human post-mortem studies. Taken together, our results suggest a contribution of neuronal STING activation to the degeneration of vulnerable neurons in ALS, establishing STING as a neuronal target. Understanding the mechanisms and consequences of this activation may provide further insight into novel STING biological function and the selectivity of neurodegeneration in ALS.

Funding Source: NIH DP2-NS106664 New York Stem Cell Foundation BJW is a New York Stem Cell Foundation - Robertson Investigator
Keywords: Neuroinflammation, amyotrophic lateral sclerosis, induced pluripotent stem cells

TOPIC: PLURIPOTENT STEM CELLS

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CELL-DERIVED EXTRACELLULAR MATRICES RAPIDLY ELICIT MATURE PHENOTYPES FROM IPSC-DERIVED SOMATIC CELLS IN 2D CULTURE

Block, Travis J. - *Technology, StemBioSys, Inc., San Antonio, TX, USA*

Abstract: Human primary cells secrete extracellular matrices in vitro that retain properties of the tissue of origin. These tissue-specific ECMs promote maturation of iPSC-derived somatic cells in 2D culture. At StemBioSys we have developed processes for reproducibly manufacturing matrices capable of promoting rapid and spontaneous maturation of iPSC-derived cardiomyocytes, neurons, hepatocytes, and beta cells in high-throughput. 7 days after seeding on cardiomyocyte maturation matrix, iPSC-derived cardiomyocytes exhibit a mature phenotype, with rod-shaped morphology, 20-30% of cells being binucleated, and dramatically unregulated cTnI expression ($p < 0.00001$). Glutamatergic neurons seeded onto an astrocyte-derived matrix rapidly cluster and begin forming synapses. After 4 days in culture, neurons on the astrocyte derived matrix have substantially more synapses relative to controls ($p < 0.01$). Hepatocytes on a stellate cell-derived matrix exhibit decreased alpha-feto-protein expression ($p < 0.01$) and increased urea secretion ($p=.08$). Moreover, iPSCs from patients with inherited diseases exhibit the disease phenotype in 2D culture. Thus, this technology has the potential

to dramatically improve the utility of iPSCs in preclinical drug testing applications for efficacy or toxicity screening.

Keywords: extracellular matrix, disease modelling, cardiomyocyte

TOPIC: NEURAL

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SIGNIFICANT THERAPEUTIC EFFECTS OF ADULT HUMAN NEURAL STEM CELLS FOR SPINAL CORD INJURY ARE MEDIATED BY MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1)

Joo, Kyeung Min - *Department of Anatomy and Cell Biologty, Sungkyunkwan University School of Medicine, Suwon, Korea*
Lee, Kyung-Hoon - *Department of Anatomy & Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea*
Kim, Chigu - *Business Development Division, Medinno Inc., Seoul, Korea*
Kim, Chung Kwon - *Department of Anatomy & Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea*

Abstract: We recently showed that adult human neural stem cells (ahNSCs) derived from hemorrhagic stroke have significant therapeutic efficacy in SCI via the replacement of damaged neural cells and pro-angiogenic effects on the microenvironment of spinal cord injury (SCI). In this study, we investigated cytokine release from ahNSCs derived from the temporal lobe of focal cortical dysplasia (FCD) type IIIa surgical samples attenuated cell death in primary spinal cord neuron cells (SCNs) by reactive oxygen species (ROS), and therapeutic efficacy for SCI using an animal model. The treatment of ahNSC-conditioned media (CM) not only reduces the viability of SCN cells by preventing H2O2-induced apoptosis but also reduced pro-apoptotic gene expressions such as cleaved caspase3 and BCL2 associated X (BAX) in SCN cells. In the SCI animal experiment, ahNSCs decreased apoptosis of SCNs in SCI lesions and improved locomotion recovery. MCP-1 was highest in the CM of ahNSCs and the addition of MCP-1 neutralizing antibody to the CM of ahNSCs reversed the neuroprotective effects of ahNSCs. In contrast, recombination protein of human MCP-1 (rhMCP1) showed neuroprotective effects, which is similar to that of the CM of ahNSCs. These data demonstrated that MCP1 released by ahNSCs have significant neuroprotective efficacy under oxidative stress in primary SCN cells and SCI animal model via alleviation of damaged neural cells by ROS.

Keywords: spinal cord injury, adult human neural stem cells, monocyte chemoattractant protein-1

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RESCUE BY EXOGENOUS ASTROCYTES OF NEURONAL GROWTH AND SYNAPTIC DEFICITS IN NEURONS CARRYING A DUPLICATION ON CHROMOSOME 16P11.2 ASSOCIATED WITH NEURODEVELOPMENTAL AND PSYCHIATRIC DISORDERS

Jiang, Xueying - *Human Genetics Branch, NIMH/NIH, Bethesda, MD, USA*
Gopalan, Krithi - *Medical School, University of Massachusetts Medical School, Boston, MA, USA*
England, Branch - *Medical School, New York University, New York, NY, USA*
Corona, Winston - *Human Genetics Branch, NIMH/NIH, Bethesda, MD, USA*
Detera-Wadleigh, Sevilla - *Human Genetics Branch, NIMH/NIH, Bethesda, MD, USA*
Williams Avram, Sarah - *Systems Neuroscience Imaging Resource, NIMH/NIH, Bethesda, MD, USA*
Usdin, Ted - *Systems Neuroscience Imaging Resource, NIMH/NIH, Bethesda, MD, USA*
McMahon, Francis - *Human Genetics Branch, NIMH/NIH, Bethesda, MD, USA*

Abstract: A 650kb duplication copy number variant (CNV) on chromosome 16p11.2 confers risk for various neurodevelopmental and psychiatric disorders. Our previous study showed that this duplication is associated with reduced neuronal counts, synaptic deficits, and incomplete differentiation of astrocytes. This project aims to explore whether the use of exogenous astrocytes can rescue these neuronal growth and synaptic deficits. 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. Mouse astrocytes were added at the beginning of neuronal differentiation in 3 carrier and 3 noncarrier lines. Live images were taken weekly. Neuron number and soma size were counted by Image J(Fiji). At 8 weeks, cells were fixed and immunostained to visualize neuronal morphology by confocal microscopy. Presynaptic (synapsin) and postsynaptic (PSD95) structures were quantified with immunocytochemistry and Vision4D. Compared to noncarriers, neurons carrying the 16p11.2 duplication showed reductions in cell counts in both neurons and astrocytes ($p< 0.01$), soma size ($p< 0.01$), dendritic complexity, and PSD95 immunoreactivity, consistent with previous studies. Carrier neurons co-cultured with exogenous astrocytes showed significanty increased neuronal cell counts, improved dendritic complexity, and increased PSD95 immunoreactivity when compared to carrier neurons alone. We conclude that exogenous astrocytes can rescue neuronal growth and synaptic deficits in neurons carrying the 16p11.2 duplication. Since astrocytes are known to play essential roles in the development and maturation of neurons and synapses, our findings suggest that some neuronal deficits associated with the 16p11.2 duplication result, at least in part, from poor growth and maturation of astrocytes. Therapeutics aimed at astrocytes may hold promise for patients with this rare but highly pathogenic CNV.

Keywords: Chromosome 16p11.2 duplication, Astrocytes, Neurodevelopment

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PROGRAMING AGE INTO HUMAN STEM CELL MODELS OF ALZHEIMER'S DISEASE

Saurat, Nathalie - *Developmental Biology, MSKCC, Long Island City, NY, USA*
Minotti, Andrew - *Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Rahman, Maliha - *Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Studer, Lorenz - *Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Abstract: Aging is the biggest risk factor for the development of Alzheimer's disease. Even patients with autosomal dominant forms of Alzheimer's disease don't develop symptoms until mid-

dle age despite having disordered APP processing throughout life. Understanding aging in the context of Alzheimer's disease is of particular interest because there is a need for new therapeutic targets for neurodegenerative diseases. We used a combination of whole genome Crispr/Cas9 screening in stem cell derived cortical neurons and transcriptomic studies to identify physiological regulators of neuronal age. Manipulation of these age-associated pathways altered Tau phosphorylation and resulted in a selective loss of viability of Alzheimer's Disease neurons. Importantly, it also induced cellular changes consistent with accelerated aging including: increased cellular senescence, loss of proteostasis, loss of heterochromatin, DNA damage and increased reactive oxygen species. This indicates that crosstalk between cellular aging pathways and disease genetics can drive neuronal loss. In addition, this study acts as a proof of principle that programming age into stem cell models of disease can enable the study of late onset neurodegenerative disease phenotypes in vitro.

Keywords: Alzheimer's Disease, Aging, Disease modeling

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MODELING SCHWANN CELL SPECIFICATION BY USING HUMAN EMBRYONIC STEM CELLS

Ramos Calcada, Raquel M. - *Institute of Anatomy, University of Zurich, Switzerland*
Varum, Sandra - *Institute of Anatomy, University of Zurich, Switzerland*
Marzorati, Elisa - *Institute of Anatomy, University of Zurich, Switzerland*
Sommer, Lukas - *Institute of Anatomy, University of Zurich, Switzerland*

Abstract: The neural crest is a transient and multipotent embryonic stem cell population that migrates throughout the embryo to populate numerous derivatives. It generates peripheral neurons and glia, smooth muscle cells of the outflow track of the heart, craniofacial bone and cartilage, and skin melanocytes. Thus, neural crest cells offer the unique possibility to study cell fate decisions during embryonic development. Schwann cells, the main glia of the peripheral nervous system, play a crucial role in neuronal support, axonal growth, myelination, as wells as regeneration of nerve and skin injury. These cells have also been implicated in several peripheral neuropathies and tumors. Despite its importance, the transcriptional program that regulates neural crest differentiation into Schwann cells remains poorly understood, contrarily to other NC-derived lineages. Furthermore, fate specification in human neural crest stem cells (hNCSCs) is largely unexplored. Here, our goal was to unravel gene regulatory networks underlying human Schwann cell specification. To accomplish this aim, we took advantage of the human embryonic stem cell (hESC) system. We efficiently differentiated hESCs into hNCSCs using a combined TGF-β and GSK-3β inhibition, followed by exposure to a glial induction medium to generate Schwann cell progenitors. Immunocytological and bulk RNA-seq analyses confirmed the generation of Schwann cell precursors and immature Schwann cells in these conditions. Intriguingly, unbiased bioinformatic analysis combined with antibody staining for primary cilia predicted ciliogenesis to be involved in Schwann cell specification. Indeed, cilia depletion in hNCSCs counteracted Schwann cell progenitor formation, demonstrating the role of ciliogenesis in human Schwann cell development. Thus, our strategy allowed us to investigate fate decisions in hNCSCs, derived from hESCs, and to identify primary cilium as a novel regulator for Schwann cell specification.

Keywords: Schwann cell, Human embryonic stem cell, Neural crest

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MATURATION DELAY OF HUMAN GABAERGIC NEUROGENESIS IN FRAGILE X SYNDROME PLURIPOTENT STEM CELLS

Loring, Jeanne F. - *Molecular Medicine, Scripps Research, Del Mar, CA, USA*
Zhang, Ai - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Sokolova, Irina - *Immunology and Microbiology, Scripps Research, La Jolla, CA, USA*
Domissy, Alain - *Center for Computational Biology, Scripps Research, La Jolla, CA, USA*
Davis, Joshua - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Rao, Lee - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Utami, Kagistia Hana - *Translational Laboratory in Genetic Medicine, National University of Singapore*
Wang, Yanling - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*
Hagerman, Randi - *MIND Institute, University of California Davis, Sacramento, CA, USA*
Pouladi, Mahmoud - *Department of Physiology, National University of Singapore*
Sanna, Pietro - *Immunology and Microbiology, Scripps Research, La Jolla, CA, USA*
Boland, Michael - *Institute for Genomic Medicine, Columbia University, New York, NY, USA*
Loring, Jeanne - *Molecular Medicine, Scripps Research, La Jolla, CA, USA*

Abstract: FXS (Fragile X Syndrome), the leading monogenic cause of intellectual disability and autism spectrum disorder, is caused by expansion of a CGG trinucleotide repeat in the 5'-UTR of the FMR1 (Fragile X Mental Retardation-1) gene. Epigenetic silencing of FMR1 results in loss of the Fragile X Mental Retardation Protein (FMRP). Most studies to date have focused on excitatory neurons, but recent evidence suggests that the GABAergic inhibitory networks are also affected. To investigate human GABAergic neurogenesis, we established a method to reproducibly derive inhibitory neurons from multiple FXS and control human pluripotent stem cell (hPSC) lines. Electrophysiological analyses suggested that the 3 FXS cell lines shared a reproducible delay in the GABA function switch, a transition in fetal development that converts the GABAA channel's function from depolarization to hyperpolarization, with profound effects on the developing brain. To investigate the cause of this delay, we analyzed 14,400 single-cell transcriptomes from FXS and control at two stages of GABAergic neurogenesis. At the earlier time point, control and FXS cells were similar, but at the later stage, FXS cells retained neuroblast proliferation-associated genes and had lower levels than controls of genes associated with action potential regulation, synapses, and mitochondria function. Our analysis suggests that loss of FMRP prolongs the proliferative stage of progenitors, which may result in more neurons remaining immature during the later stages of neurogenesis. This could have profound implications for homeostatic excitatory-inhibitory circuit development in

FXS, suggests a novel direction for understanding disease mechanisms, and may help to guide therapeutic interventions.

Funding Source: This work was funded by the NIH (R33MH087925) and CIRM (TR3-05603, RT3-07655 DISC2-09073, DISC2P-11595, TR1-01250, RT1-01108, GC1R-06673-A). We thank the Scripps Research Skaggs Graduate School for supporting A.Z.
Keywords: Fragile X Syndrome, GABAergic neurons, Autism

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HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS REVEAL PROGENITOR PATHOLOGY IN EML1-LINKED CORTICAL MALFORMATION

Jabali, Ammar A. - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Hoffrichter, Anne - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Uzquiano, Ana - *Department of Stem Cell and Regenerative Biology, Harvard, Boston, MA, USA*
Marsoner, Fabio - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Wilkens, Ruven - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Siekmann, Marco - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Bohl, Bettina - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Rossetti, Andrea - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Horschitz, Sandra - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Koch, Philipp - *HITBR, Central Institute of Mental Health, Mannheim, Germany*
Francis, Fiona - *INSERM, Institut du Fer à Moulin, Paris, France*
Ladewig, Julia - *HITBR, Central Institute of Mental Health, Mannheim, Germany*

Abstract: The human cerebral cortex is a highly organized structure, with layers and folds, which are formed in still little-understood ways. Malformations associated with defects in human cortical development (MCD) can result in cortical disorganization with severe consequences including epilepsy and intellectual disability. Various mouse models have been used to study human MCD, but they are limited by structural differences between the murine and the human brain. To overcome this species-specific limitation, we used cerebral organoids derived from patients and genome edited induced pluripotent stem cells to address pathophysiological changes associated with a complex MCD caused by mutations in the Echinoderm microtubule-associated protein-like 1 (EML1) gene. EML1-deficient organoids display ectopic neural rosettes at the basal side of the ventricular zone areas and clusters of heterotopic neurons. Single-cell RNA sequencing shows an upregulation of basal radial glial (RG) markers and human-specific extracellular matrix components in the ectopic cell population. Gene ontology and molecular analyses suggest that ectopic progenitor cells originate from perturbed apical RG cell behavior, including significant changes in the cleavage angle of EML1-deficient cells, reduced length as well as perturbed structures of their primary cilia in addition to yes-associated protein 1 (YAP1) triggered expansion. Our data highlight a progenitor origin of EML1-mutation induced MCD and provide new mechanistic insight into the human disease pathology suggesting that organoid-based systems serve as promising models to study early human cortical development and associated disorders.

Funding Source: ERA-NET NEURON, JTC 2015 Neurodevelopmental Disorders, STEM-MCD German research foundation (DFG), Project LA 2933/2-1 Hector Stiftung II
Keywords: EML1 mutation, cerebral organoids, malformation of cortical development

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HUMAN BRAIN ORGANOIDS REVEAL ASYNCHRONOUS DEVELOPMENT OF CORTICAL NEURON CLASSES AS A SHARED FEATURE OF AUTISM

Velasco, Silvia - *Cell Biology, Murdoch Children's Research Institute, Parkville, Victoria, Australia*
Paulsen, Bruna - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*
Kedaigle, Amanda - *Stanley Center, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA*
Pigoni, Martina - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*
Adiconis, Xian - *Stanley Center, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA*
Uzquiano, Ana - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*
Sartore, Rafaela - *Stanley Center, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA*
Yang, Sung - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*
Symvoulidis, Panagiotis - *Department of Biological Engineering, MIT, Cambridge, MA, USA*
Boyden, Edward - *Department of Biological Engineering, MIT, Cambridge, MA, USA*
Regev, Aviv - *Department of Biology, MIT, Cambridge, MA, USA*
Levin, Joshua - *Stanley Center, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA*
Arlotta, Paola - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*

Abstract: Autism spectrum disorder (ASD) is a childhood-onset neurodevelopmental disorder characterized by cognitive, motor, and sensory deficits. Despite hundreds of genes have been associated with risk for ASD, the alterations in the human brain caused by mutation in these genes remain unclear. Here, we leveraged reproducible organoid models of the human cerebral cortex to identify cell type-specific developmental abnormalities resulting from haploinsufficiency in three ASD-risk genes: SUV420H1/KMT5B, ARID1B, and CHD8. By combining single-cell RNA-sequencing, single-cell ATAC-sequencing and proteomic analysis of individual ASD mutant and control organoids, we find that the three ASD-risk genes converge on a phenotype of asynchronous development of two main cortical neuronal lineages, GABAergic neurons and deep-layer excitatory projection neurons. Although these phenotypes are consistent across cell lines, their expressivity is influenced by different genomic contexts, resembling the variable clinical manifestations observed in individuals. Interestingly, SUV420H1, ARID1B and CHD8 haploinsufficiency affect similar developmental processes through distinct molecular targets, suggesting that the shared clinical pathology of these genes may derive from higher-order processes of neuronal differentiation and circuit wiring. This work shows that reproducible

cortical organoids, combined with high-throughput single cell genomic methods, are invaluable systems for unbiased identification of cell type-specific neurodevelopmental abnormalities and paves the way for further analysis of a larger spectrum of ASD-risk genes in organoids, to understand how they converge on the neurobiology of this multifaceted disorder.

Keywords: Autism, Brain organoids, Neurodevelopment

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GENOME-WIDE CRISPR-INTERFERENCE SCREEN IDENTIFIES NEURONAL GENES WHOSE KNOCKDOWN ARE PROTECTIVE AGAINST MICROGLIAL INFLAMMATION

Chang, Jonathan K. - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Alhambra, CA, USA*
Ichida, Justin - *Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Cognitive and behavioral issues in patients with neurodegenerative disease represent a significant public health burden for an aging global population, and there are currently no effective treatments. Though the initial activation of microglia – the tissue-resident immune cell in the brain – is considered a beneficial response to remove danger signals and pathogens from the central nervous system, this activation is insufficient in fully restoring neuronal homeostasis. This leads to an unresolved microglial activation state and subsequently a chronic inflammatory environment, one improper for maintaining neuronal health and ultimately contributes to cognitive decline. As such, targeting microglial inflammation becomes an attractive therapeutic strategy to enhance long-term neuronal health. Current strategies aimed at reducing microglial inflammation, however, have remained largely ineffective. This may in part be due to the preclusion of downstream beneficial contributions. Instead of reducing inflammation, we asked: how can we protect neurons from inflammatory microglia? Using a novel tri-culture system of induced pluripotent stem cell-derived neurons, microglia, and primary astrocytes, we showed that microglia, when activated by lipopolysaccharide and adenosine triphosphate, drastically kill neurons in vitro. We then performed a genome-wide CRISPR-interference screen to identify genes for which knockdown was protective against microglial inflammation-induced toxicity. Intriguingly, we found knockdown of multiple genes related to prostaglandin signaling to be significantly protective. These results suggest that targeting prostaglandin signaling may help to limit neurodegeneration in the presence of inflammatory microglia. Completion of this study will identify more selective targets within prostaglandin signaling pathways that may have substantially more therapeutic success in the clinic.

Funding Source: Rainwater Charitable Foundation
Keywords: Microglia, Neuroinflammation, CRISPR-interference screen

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GENERATION OF MATURE BRAIN ASSEMBLOIDS FOR MODELING DEVELOPMENT OF SCHIZOPHRENIC BRAIN

Kim, Eunjee - *Biological Sciences, Seoul National University, Seoul, Korea*
Hong, Soojung - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Kim, Joung-Hun - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Kim, Sanguk - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Shin, Kunyoo - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*

Abstract: The biggest challenge to study human brain is the lack of experimental systems that can precisely represent neurodevelopmental processes. Here we developed two-step, mature brain assembloids mimicking early and late stage of human brain development. We firstly generated early-stage organoids by increasing the activity of Hh and Wnt signaling pathway, which induced expanded neural progenitor cell (NPC) layer. We then developed late-stage brain organoids, called mature brain 'assembloids' by reconstituting the outermost reelin-expressing layer for neuronal guidance and integrating glial cell population, which showed mature cortical architecture and synaptic function. Using our early and late stage brain assembloids, we elucidated two-step, multi-factorial mechanisms for the development of human schizophrenic brain. We identified that FOXO1 and NFIA epigenetically suppress target gene networks related to cell proliferation and upregulate networks related to cell differentiation, respectively, which leads to the early differentiation of NPCs at the early stage of schizophrenia development. Furthermore, we found that interactions with schizophrenic neurons and subsequent changes in glial cells play a critical role in late stage schizophrenia development. We showed that neuron-derived TGFβ1 and IL-32 induces transcriptomic changes of glial cells, which leads to astrocytic secretion of TSP1 and microglial secretion of TNFα. These factors promote astrocytic remodeling of postsynaptic density and microglial induction of neuronal apoptosis, which implicates synaptic defects at the late stage schizophrenia development. Taken together, our data show that two-step brain assembloids can recapitulate development of human brain and complex neurological diseases.

Keywords: Brain organoids, Schizophrenia, Neurodevelopmental disorder

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GENERATING CEREBELLAR ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

Ryu, Seungmi - *NIH, NCATS, Rockville, MD, USA*
Hong, Hyenjong - *NCATS, NIH, Rockville, MD, USA*
Jovanovic, Vukasin - *NCATS, NIH, Rockville, MD, USA*
Tristan, Carlos - *NCATS, NIH, Rockville, MD, USA*
Simeonov, Anton - *NCATS, NIH, Rockville, MD, USA*
Singec, Ilyas - *NCATS, NIH, Rockville, MD, USA*

Abstract: The cerebellum plays an important role in motor coordination and is affected in various neurological conditions. Most of our current understanding of human cerebellum is derived from postmortem tissues, animal models, and immortalized cell lines cultured in conventional 2D cultures. Over the past decade, hu-

man induced pluripotent stem cells (iPSCs) have become increasingly popular for generating various 3D organoid models. However, little is known about how to generate bona fide cerebellar tissue from iPSCs. Here, we developed a novel 60-day organoid differentiation strategy that recapitulates cerebellum-specific developmental processes. We demonstrate the generation of two distinct primordial regions that resemble the rhombic lip and cerebellar plate ventricular zone. Immunostainings and Western blot analysis confirmed that these primordial regions give rise to major neuronal cell type of the cerebellum (i.e. Purkinje cells, granule cells, interneurons) as well as glial cells resembling Bergmann glia that are specialized to guide migratory granule cells to their final destination. Remarkably, self-organizing cerebellar organoids formed the appropriate layers of the cerebellum and neuronal migration followed an outside-in pattern, which contrasts with telencephalic brain regions and organoids that follow an inside-out migration pattern. To demonstrate utility for disease modelling, cerebellar organoids were generated using iPSCs derived from patients with Friedreich's ataxia, an autosomal-recessive neurogenerative disorder caused by a defect in the frataxin gene. Ongoing molecular, cellular, and electrophysiological studies are aimed at elucidating disease phenotypes and testing therapeutic modalities including drug screening and gene editing with CRISPR-Cas9. In summary, our organoid model provides new opportunities to study the cerebellum in basic and translation research with potential future applications in regenerative medicine.

Keywords: organoid, cerebellum, Friedreich's ataxia

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EVALUATING NEURONAL, SYNAPTIC, AND NETWORK FUNCTION IN STEM CELL MODELS OF NEURAL DEVELOPMENT AND DISEASE

Hayes, Heather - *Applications Development, Axion BioSystems, Atlanta, GA, USA*
Chvatal, Stacie - *Applications Development, Axion BioSystems, Atlanta, GA, USA*
Clements, Mike - *Marketing, Axion BioSystems, Atlanta, GA, USA*

Abstract: Induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Further, advances in genetics and proteomics have accelerated neural disease-in-a-dish models, while 3D cell preparations, such as organoids, have enabled models that better recapitulate human development. To optimize, validate, and utilize complex in vitro neuronal models, it is critical to characterize the function of neurons, synapses, and networks over relevant time courses. Here, we present data supporting the use of label-free, multi-well microelectrode array (MEA) technology as an efficient approach for reliable, repeated quantification of cellular and network function across development and in disease. First, the Maestro multi-well MEA system (Axion BioSystems) was used to characterize human iPSC-derived cortical organoid function across 10 months in vitro. Organoids exhibited increasingly complex spiking activity over time, with network events becoming more frequent. Local field potentials were also compared to electroencephalograms from premature neonates, revealing that the development of complex network oscillations in cortical organoids mimic and predict the network dynamics of early human brain development. In addition, we also validated two human iPSC disease-in-a-dish models. In a model of Dravet syndrome, patient-specific hiPSC-derived neurons were tracked for 36 days in vitro. Dravet syndrome cultures exhibited significantly higher mean firing rate, network burst duration, and network burst density compared to wild type controls, reflecting an epileptic phenotype. In a model of Fragile X syndrome, hiPSC-derived neurons exhibited reduced FMR1 mRNA and FMRP expression compared to isogenic controls. Reduced expression corresponded to increased neural activity after DIV21. The disease phenotype was reversed with addition of FMR1 mRNA or co-culture with control neurons, both in a dose-dependent manner. By bringing human biology to a dish, hiPSC-derived neurons deliver biologically-relevant models for studying neural development and disease. The Maestro multi-well MEA platform enables label-free chronic tracking of neural network function ideal for validating neuronal models and developing novel therapeutics.

Keywords: Neurodevelopment, Organoids, Epilepsy

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ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NEURONS MODELING NEUROLOGICAL DISEASES USING HIGH-DENSITY MICROELECTRODE ARRAYS

Duperrex, Eliane - *MaxWell Biosystems AG, Zurich, Switzerland*
Magnani, Dario - *Early Discovery, Charles River Laboratories, Saffron Walden, UK*
Ronchi, Silvia - *MaxWell Biosystems AG, Zurich, Switzerland*
Butler, Larissa - *Early Discovery, Charles River Laboratories, Saffron Walden, UK*
Jäckel, David - *MaxWell Biosystems AG, Zurich, Switzerland*
Iovino, Mariangela - *Early Discovery, Charles River Laboratories, Saffron Walden, UK*
Burley, Russell - *Early Discovery, Charles River Laboratories, Saffron Walden, UK*

Abstract: The emergence of induced-pluripotent-stem-cell (iPSC) technology paved the way for in-vitro characterization of human neurons. The novel 'forward programming' technique consists of inducing gene expression to promote the cell fate into the desired cell type, thus promoting a fast and reproducible cell differentiation. In parallel, extracellular electrophysiology platforms have allowed monitoring entire human-neuron populations at unprecedented resolution over weeks. In this work, we studied the electrophysiology of healthy and disease lines of glutamatergic neurons generated via forward programming (bit.bio, Cambridge, UK), using high-density microelectrode arrays (HD-MEAs, MaxWell Biosystems, Zurich, Switzerland). We used a disease line carrying a single nucleotide mutation (M337V) in the TARDBP gene to model amyotrophic lateral sclerosis (ALS), and by insertion of 50 CAG repeats in the HTT gene, another disease line to model Huntington disease (HD). Healthy and disease lines were cultured on 6-well-plate HD-MEAs over >4 weeks, and recordings were performed twice a week. We extracted electrophysiological metrics such as neuron firing rate, action potential amplitude, and interspike interval. Additionally, we characterized the extension of the axonal arborization of single neurons and computed the axonal conduction velocity. We found that firing rate and interspike interval differed significantly between healthy, ALS and HD glutamatergic neuron cultures at specific developmental stages . Additionally, we characterized how the three lines matured and developed over time. In summary, this work demonstrates that the combination of iPSC and HD-MEA technologies allows to successfully characterize healthy and diseased neurons and to identify their phenotypical differences.

Keywords: microelectrode arrays, neurodegenerative diseases, iPSC-derived neurons

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TRANSPLANTATION OF HUMAN GLIAL PROGENITOR CELLS INTO ADULT HUNTINGTON DISEASE MICE IMPROVES NEURONAL ARCHITECTURE AND ASPECTS OF BEHAVIOR

Benitez Villanueva, Carlos - *Center for Translational Neuromedicine, University of Copenhagen and University of Rochester, Cambridge, MA, USA*
Tate, Ashley - *CNS group, Sana Biotechnology, Cambridge, MA, USA*
Huynh, Gwen - *Center for Translational Neuromedicine, University of Copenhagen, Denmark*
Lorenzen, Signe - *Brainstem, University of Copenhagen, Denmark*
Benraiss, Abdellatif - *Center for Translational Neuroscience, University of Rochester, NY, USA*
Goldman, Steven - *Center for Translational Neuroscience, University of Rochester, NY, USA*

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat within the HTT gene. It is characterized by motor dysfunction, striatal degeneration, and hyperexcitability in the setting of a loss of synaptic homeostasis. Glial pathology is a significant contributor to disease pathogenesis in HD, and we have found that neonatal replacement of diseased with healthy glia can delay disease onset and progression in mouse models of HD. Here we asked if transplanting normal human glial progenitor cells (hGPCs) into adult HD mice might similarly ameliorate disease progression. We found that the introduction of hGPCs into the striata of adult R6/2 HD mice significantly ameliorated both their motor deficits, as determined by rotarod and open-field ambulatory activity, and their behavioral phenotype, as assessed by novel object recognition and elevated plus maze testing; furthermore, the median survival of hGPC-engrafted R6/2 mice was significantly longer than untreated controls. To better understand the underlying mechanisms of this improvement, we collected neuronal nuclear single cell RNA-seq data from the striatal medium spiny neurons of transplanted and unengrafted control mice; we also investigated transplant-associated structural changes to their dendritic trees. To this end, we used retrograde labeling of striatal MSNs with glycoprotein-deleted rabies virus in hGPC-engrafted and control adult R6/2 mice, and then used deconvolution confocal microscopy followed by Sholl analysis to map the dendritic arbors of the tagged MSNs. By this means, we found that the dendritic architecture of striatal MSNs was significantly restored in the hGPC-engrafted mice, and led to a significant increase in their dendritic complexity and synaptic maturity. These findings suggest that glial replacement may delay disease progression in HD, and that this is associated with the dynamic reorganization of medium spiny neuron dendritic architecture.

Funding Source: Support by the Novo Nordisk and Lundbeck Foundations and CHDI
Keywords: Huntington's Disease, Glial Progenitor Cells, Medium Spiny Neurons

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DEVELOPMENT OF AN ORGAN-ON-CHIP SYSTEM TO STUDY PARKINSON DISEASE

Otero, Maria G. - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Laperle, Alex - *RMI, Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA*
Sances, Sam - *Insitro, San Francisco, CA, USA*
Behar, Moris - *RMI, Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA*
Ondatje, Briana - *RMI, Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA*
Spivia, Weston - *Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Van Eyk, Jennifer - *Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Svendsen, Clive - *RMI, Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA*

Abstract: Parkinson disease (PD) is complex neurodegenerative disorder in which nigral dopaminergic neurons projecting to the striatum are progressively lost, leading to motor and non-motor consequences. The cause of neuronal death remains unknown, though both genetic and environmental factors may be involved. Diagnosis usually occurs following physical manifestations, at which point there is already substantial death of dopamine neurons. Discovering biomarkers for early detection of disease could greatly benefit the treatment course. Organ-on-chips permit the in vitro co-culture of different cell types, to faithfully recapitulate brain physiology and different aspects of a disease. We have developed an organ-on-chip model incorporating patient-specific induced pluripotent stem cell (iPSCs) to study sporadic young onset PD. The Chip (Emulate Inc.) has two channels to culture independent cell types, separated by a porous membrane. For the PD-Chip, the top channel contains patient-specific iPSC-derived dopaminergic neurons and the bottom contains iPSC-derived brain microvascular endothelial cells (BMECs). The porous membrane acts as a blood brain barrier. The primary goal of this project is to establish robust and reproducible organ-on-chip systems to study sporadic young onset PD by identifying early metabolomic, transcriptomic and proteomic biomarkers. Preliminary data shows that iPSC-derived dopaminergic neurons and BMECs survive in the PD-Chip for 28 days, with no sign of neuronal toxicity measured by a Lactate Dehydrogenase cytotoxicity assay. Immunostaining with specific cellular markers shows tyrosine hydroxylase (TH)-positive dopamine neurons and Glucose transporter-1 (Glut-1)-positive BMECs, and single nuclei RNA-sequencing further demonstrates the presence of multiple cell types. We are currently analyzing transcriptomics and metabolomics for the PD-Chip. Moreover, we are optimizing the culture of dopaminergic and medium spiny neurons on the PD-Chip to model the nigral-striatal pathway. In addition to organ-on-chips, we are developing organoid and assembloid models of PD. Using PD-Chip and organoid/assembloid systems will hopefully elucidate mechanisms underlying sporadic young onset PD, and reveal early biomarkers.

Funding Source: 5UG3NS105703-02
Keywords: Parkinson Disease, Organ-On-Chip, Organoids

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DEFINING THE ROLE OF KAT6A IN HUMAN NEURODEVELOPMENT AND ARBOLEDA-THAM SYNDROME

Nava, Aileen A. - *Human Genetics, Computational Medicine, and Pathology/Laboratory Medicine, University of California, Los Angeles, CA, USA*
Arboleda, Valerie - *Human Genetics, Computational Medicine, and Pathology/Laboratory Medicine, UCLA, Los Angeles, CA, USA*

Abstract: Lysine (K) acetylation is required for normal brain development and pathogenic mutations in enzymes that acetylate K residues on histone tails, termed K acetyltransferases (KATs) are enriched in neurodevelopmental disorders (NDDs). Arboleda-Tham Syndrome (OMIM#616268) is caused by de novo, truncating mutations in KAT6A and patients manifest with early developmental delays, intellectual disability, language deficits, microcephaly, cardiac abnormalities, and craniofacial defects. Clinical studies have demonstrated that late-truncating mutations localized to exons 16 and 17 are correlated with more severe developmental delay and increased risk of microcephaly and cardiac defects. The molecular mechanisms underlying this genotype-phenotype relationship in Arboleda-Tham Syndrome have not been identified. In humans, it is unclear how KAT6A influences the cellular behavior, differentiation potential, and fate of neural lineages. To answer these questions, we differentiated induced pluripotent stem cells (iPSC), derived from either Arboleda-Tham Syndrome patients (n=2) or matched controls (n=2), into cortical organoids (COs) under chemically defined conditions and performed transcriptomic profiling and immunofluorescence at three timepoints during CO differentiation. Biological samples were collected in triplicate at three timepoints during differentiation. Our data shows that the KAT6A mutation leads to significant transcriptomic dysregulation (FDR< 0.05) of 1,931 genes in iPSCs, 1,673 genes in day 15 COs, and 4,088 genes in day 25 COs. These significant differentially expressed genes (sigDEGs) are enriched for processes ranging from regulation of chromatin confirmation to neural tube patterning/development. Remarkably, unsupervised hierarchical clustering of the normalized gene count z-scores from these sigDEGs revealed that early and late KAT6A mutations function through both shared and mutation-specific transcriptomic mechanisms that may explain the variable manifestation observed in patients. Upon completion of our study, our findings will shed light on the role of KAT6A in human neurodevelopment, stem cell biology, and Arboleda-Tham Syndrome—thereby bringing us closer to identifying therapeutic targets for this rare disorder and potentially other related NDDs.

Funding Source: Aileen A. Nava acknowledges the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Training Program for supporting her stipend during the generation and analyses of this data/research.
Keywords: KAT6A (LYSINE ACETYLTRANSFERASE 6A), ARBOLEDA-THAM SYNDROME, neurodevelopment

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CSF EXOSOMAL MIRNAS IMPACT ON NEURAL STEM CELL DIFFERENTIATION CONTRIBUTING TO A PREMATURE NEURO-GLIAL SWITCH FOLLOWING HAEMORRHAGE IN THE DEVELOPING BRAIN

Gialeli, Andriana - *School of Medicine and Health Sciences, Carl Von Ossietzky University of Oldenburg, Bristol, UK*
Spaull, Robert - *Regenerative Medicine Lab, Bristol Medical School, University of Bristol, UK*
Plösch, Torsten - *Perinatal Neurobiology Lab, School of Medicine and Health Sciences, Carl von Ossietzky University of Oldenburg, Germany*
Uney, James - *Regenerative Medicine Lab, Bristol Medical School, University of Bristol, UK*
Cordero-Llana, Óscar - *Regenerative Medicine Lab, Bristol Medical School, University of Bristol, UK*
Heep, Axel - *Perinatal Neurobiology Lab, School of Medicine and Health Sciences, Carl von Ossietzky University of Oldenburg, Germany*

Abstract: The microenvironment of the neural stem/progenitor cells (NSPCs) can influence their fate during cortical development. Cerebrospinal fluid (CSF), that runs along the lateral ventricle walls, provides factors which support the division, development and migration of the surrounding NSPCs. Alterations in CSF composition (growth factors, hormones, miRNAs) after a brain injury can cause the deregulation of NSPCs homeostasis and contribute to central nervous system implications. While there are many studies about adult NSPCs-CSF interaction after an intraventricular haemorrhage (IVH) in adults, less is known about this interaction during development. We hypothesised that the miRNA CSF's content may alter after IVH, and this can affect NSPCs fate. We tested 2.083 miRNAs in the CSF from 10 preterm infants with intraventricular haemorrhage (IVH-CSF) by HTG EdgeSeq miRNA whole transcriptome assay. We identified 587 differentially expressed miRNAs (DESeq tool, Reveal Software) and 9 miRNA families with at least three differentially expressed family members (miRViz tool). The deregulated miRNAs are involved in processes such as cell survival, proliferation and differentiation, metabolism, inflammation and reactive gliosis. In functional in vitro assays, we showed that exosomal miRNAs extracted from IVH-CSF shifted the differentiation of human fetal neural stem cells towards astrocytes. These results provide first insight into potential mechanisms of CSF-NSPCs interactions during brain development guiding reactive gliosis following perinatal brain injury.

Funding Source: The project was funded by Carl von Ossietzky University of Oldenburg and the Castang Foundation.
Keywords: neural progenitor cells, cerebrospinal fluid, micro RNA

TOPIC: PANCREAS

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ASSESSING COMBINATION THERAPIES IN HUMAN PANCREATIC CANCER ORGANOIDS USING A STANDARDIZED SCREENING WORKFLOW

Brandenberg, Nathalie - *Business Development, SUN bioscience, Lausanne, Switzerland*
Roch, Aline - *Research and Development, SUN bioscience, Lausanne, Switzerland*
Kuttler, Fabien - *Biomolecular Screening Facility, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Turcatti, Gerardo - *Biomolecular Screening Facility, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Homicsko, Krisztian - *Department of Oncology, Ludwig Institute for Cancer Research, Lausanne, Switzerland*
Hoehnel, Sylke - *Research and Development, SUN bioscience, Lausanne, Switzerland*

Abstract: Precision medicine for cancer patients promises the tailoring of targeted therapies to specific genetic alterations. Currently, alterations in 43 oncogenes can be targeted based on Level 1 clinical evidence. Still, the majority of cancer patients lack efficient targeted therapy options with lasting benefit. Ex vivo assays, such as tumor tissue explants, hold the promise to directly measure the impact of anticancer compounds and their combinations. However, a significant challenge for ex vivo drug testing lies in the efficient establishment of fresh primary cell cultures for testing, within clinically actionable timeframe, and in the available tumor volume. To this end, patient-derived organoids (PDOs) have been proposed as viable and efficient alternatives for ex vivo testing. PDOs show long-term expansion potential while retaining tumor histopathology as well as cancer gene mutations. We have shown how homogenous reproducible PDOs based on Gri3D® hydrogel microwell arrays could be generated for high-throughput drug testing of single and combination therapies. Here we demonstrate on human pancreatic cancer organoids how amalgamation of anti-cancer drugs could enhance efficacy compared to mono-therapy approaches. By targeting pathways in a characteristically synergistic or an additive manner, a lower therapeutic dosage of each individual drug is required, potentially also reducing toxic side effects.

Funding Source: Tumour samples were obtained from the Department of Gastroenterology and Hepatology, Centre Hospitalier Universitaire Vaudois under ethical approval from the Ethics Committee of the Canton Vaud, Switzerland (CER-VD: 2017-00359).
Keywords: Organoids, Cancer, Screening

TOPIC: PLURIPOTENT STEM CELLS

400

SINGLE-CELL TRANSCRIPTOMICS CAPTURES FEATURES OF HUMAN MIDBRAIN DEVELOPMENT AND DOPAMINE NEURON DIVERSITY IN BRAIN ORGANOIDS

Fiorenzano, Alessandro - *Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Three-dimensional brain organoids have emerged as a valuable model system for studies of human brain development and pathology. Here we establish a midbrain organoid culture system to study the developmental trajectory from pluripotent stem cells to mature dopamine neurons. Using single cell RNA sequencing, we identify the presence of three molecularly distinct subtypes of human dopamine neurons with high similarity to those in developing and adult human midbrain. However, despite significant advancements in the field, the use of brain organoids can be limited by issues of reproducibility and incomplete maturation which was also observed in this study. We therefore designed bioengineered ventral midbrain organoids supported by recombinant spider-silk microfibers functionalized with full-length human laminin. We show that silk organoids reproduce key molecular aspects of dopamine neurogenesis and reduce inter-organoid variability in terms of cell type composition and dopamine neuron formation.

Keywords: human midbrain organoids, single-cell transcriptomics, Parkinson´s disease

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RECONSTRUCTION OF HUMAN BRAIN TISSUE IN VITRO TO DISSECT THE MECHANISMS UNDERLYING SYNUCLEIN PATHOLOGY

Mesentier-Louro, Louise A. - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Goldman, Camille - *Neuroscience, Icahn Medical School at Mount Sinai, New York, NY, USA*
R'Bibo, Lea - *Neuroscience, Icahn Medical School at Mount Sinai, New York, NY, USA*
Frank, Alexander - *Neuroscience, Icahn Medical School at Mount Sinai, New York, NY, USA*
Blanchard, Joel - *Neuroscience, Icahn Medical School at Mount Sinai, New York, NY, USA*

Abstract: Intracellular aggregation of synuclein is a hallmark of Parkinson's disease (PD), dementia with Lewy bodies (LBD), and multiple system atrophy (MSA). Findings from animal models of synucleinopathies have not successfully translated to human disease, and conventional 2D cell culture systems fail to recapitulate the complex cellular interactions in the human brain. As a result, we have limited insight into the mechanisms that govern the initiation and severity of synuclein pathologies in PD, LBD, and MSA, hindering the development of effective therapeutics and diagnostics. To address this, we developed the multi-cellular integrated brain (miBrain), an iPSC-derived human brain tissue that contains anatomically correct cerebrovasculature, physiologically active neuronal circuits, myelination, and a neuroinflammatory system. Vascular networks present in the miBrain expressed platelet endothelial cell adhesion molecule and were associated to platelet-derived growth factor receptor beta-expressing peri-

cytes and S100B-expressing astrocytes, forming a functional blood-brain barrier. Neurons within the miBrain expressed neurofilament and were enwrapped by myelin basic protein expressed by oligodendrocytes. TMEM119-expressing microglia are present throughout the miBrain tissue in a grid-like pattern similar to the human brain. We applied the miBrain to dissect cell-autonomous and non-cell-autonomous mechanisms underlying alpha-synuclein pathology, including synuclein aggregation, accumulation, and neurotoxicity. Using live imaging, we observed that iPSC-derived astrocytes readily take up synuclein monomer, which leads to intracellular aggregation and accumulation. Mitochondrial dyes revealed that synuclein uptake coincided with changes in mitochondrial morphology and increased superoxide levels. These results demonstrate that the miBrain system can replicate synuclein pathology observed in human brain tissue. We are further developing the model and applying it to dissect the molecular mechanisms underlying genetic risk factors associated with synuclein pathology.

Keywords: miBrain, synuclein, Parkinson's disease

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INDUCED PLURIPOTENT STEM CELLS AND HIGH QUALITY ISOGENIC CONTROL LINES - THE NEXT STEP IN THE MODELLING OF GENETIC DISEASES

Jadali, Azadeh - *Stem Cell Services, Infinity BiologiX, Piscataway, NJ, USA*
Moore, Jennifer - *Cellular Services, Infinity BiologiX, Piscataway, NJ, USA*

Abstract: As sequencing technologies become faster, less expensive and more powerful, the ability to identify genetic causes of disease has become easier. Despite the advances made in identifying these genetic components, understanding their exact molecular mechanisms and developing potential treatment options remains difficult for a number of reasons, particularly because the human population is so diverse. Since their initial generation in 2007, human induced pluripotent stem cells (hiPSC) have been used to model human development, disease progression, and for drug screening and for toxicology testing. It is now possible to reprogram a wide range of cell types allowing a greater diversity of hiPSC to be generated and increasing the availability of hiPSC containing specific mutations. These hiPSC provide an excellent tool for continuing to understand and develop treatments for genetic diseases – they are human, they can be differentiated into any cell type, and they can be used in a relatively high-throughput manner. The use of the CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas system can create isogenic cell lines that will serve as better controls and help eliminate effects that are due to genetic variance rather than a biological mechanism. Infinity BiologiX hosts and maintains the NINDS Cell and Human Data Repository (NHCDR) (https://bioq.nindsgenetics.org/) and the NIMH Repository & Genomics Resource (https://www.nimhgenetics.org/). These repositories house iPSC, edited iPSC, fibroblasts and cryopreserved lymphocytes from more than 1000 subjects, including a GMP grade iPSC cell line. In conjunction with these repositories, we have developed high throughput, cost efficient workflows for generating hiPSC from many different types of source cells and using CRISPR/Cas9 to genetically modify these cell lines. These cell lines are available to academic and for-profit researchers worldwide to further the study and treatment of human genetics, development, and disease.

Funding Source: U24NS095914 – 06 (NINDS) and U24 MH068457-19 (NIMH)
Keywords: induced pluripotent stem cells, Edited iPSCs, Repository

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HUMAN PLURIPOTENT STEM CELL-DERIVED MACROPHAGES FOR MODELING MYCOBACTERIAL INFECTION

Sun, Shicheng - *Murdoch Children's Research Institute, University of Melbourne, Australia*
See, Michael - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Nim, Hieu - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Ng, Elizabeth - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Hidalgo-Gonzalez, Alejandro - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Ramialison, Mirana - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Elefanty, Andrew - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Sutton, Philip - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Sarkar, Sohinee - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Stanley, Ed - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*

Abstract: Human macrophages are a natural host of many mycobacterium species, including Mycobacterium abscessus (M. abscessus), an emerging pathogen affecting patients with lung diseases and immunocompromised individuals. There are few available treatments and the search for effective antibiotics against M. abscessus has been hindered by the lack of a tractable in vitro intracellular model of infection. Here, we established a reliable model for M. abscessus infection using human pluripotent stem cell-derived macrophages (hPSC-macrophages). hPSC differentiation permitted a reproducible generation of functional human macrophages that were highly susceptible to M. abscessus infection. Electron microscopy demonstrated that M. abscessus was present in the vacuoles of hPSC-macrophages. RNA-sequencing analysis revealed a time-dependent immune response to M. abscessus with different gene expression patterns. Engineered tdTOMATO-expressing hPSC-macrophages with GFP-expressing M. abscessus enabled rapid image-based analysis of intracellular infection and quantitative assessment of antibiotic resistance. Our study describes the first hPSC-based model for M. abscessus infection, which represents a novel platform for studying M. abscessus-host interaction and an accessible tool for drug discovery.

Keywords: Macropahges, Mycobacterial infection, High throughput analysis

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EVALUATION OF WNT/B-CATENIN SIGNALING PATHWAY IN EUPLOID AND TRISOMY 21 IPSCS

Prutton, Kendra M. - *Toxicology, University of Colorado Anschutz, Denver, CO, USA*
Marentette, John - *Toxicology, University of Colorado, Aurora, CO, USA*
Roede, James - *Toxicology, University of Colorado, Aurora, CO, USA*
Ward, Alexandra - *Toxicology, University of Colorado, Aurora, CO, USA*

Abstract: Down syndrome (DS) is the most common human aneuploidy, occurring in approximately 1 in 700 live births. Based upon previous reports and observations in our DS iPSCs showing slowed proliferation and spontaneous differentiation, we investigated the canonical Wnt/β-catenin signaling pathway in an isogenic model system using acute and 24hr CHIR99021 (CHIR) treatment. Here, we show increased baseline expression of several canonical Wnt pathway genes, such as FZD1/2, and increased protein abundance of nuclear β-catenin in our DS iPSCs. We found that several germ layer markers are differentially expressed following acute and 24hr CHIR treatment. DS iPSCs have decreased mitochondrial respiration compared to isogenic euploid, controls and DS and euploid iPSC maximal oxygen consumption rate responded differentially following an acute CHIR treatment. Furthermore, acute exposure to CHIR significantly increased glycolysis, glycolytic capacity, and glycolytic reserve in euploid iPSCs. However, DS iPSCs were unresponsive to the same exposure. Our results demonstrate dysfunction of the Wnt signaling pathway in DS, which can lead to DS-specific phenotypes, particularly those involved in neurodevelopment and neurodegeneration.

Keywords: Down syndrome, Wnt/B-catenin pathway, Mitochondrial function

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CONFOUNDING IN IPSC-BASED MODELS OF DIAMOND-BLACKFAN ANAEMIA

Rani, Sonam - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Nandy, Krittika - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Chaudhury, Anurag - *Department of Haematology, Christian Medical College, Vellore, India*
NA, Fouzia - *Department of Haematology, Christian Medical College, Vellore, India*
George, Biju - *Department of Haematology, Christian Medical College, Vellore, India*
Velayudhan, Shaji - *Centre for Stem Cell Research and Department of Haematology, Christian Medical College, Vellore, India*

Abstract: Diamond-Blackfan anemia (DBA) is a rare ribosomopathy characterized by defective ribosome biogenesis resulting in selective perturbation in red blood cell differentiation causing severe anemia in children. Mutations in several ribosomal proteins (RP) have been reported in DBA manifestation, where RPS19 mutations account for >20% of the DBA cases. To understand the underlying mechanism of the selective erythroid failure, we generated iPSCs (induced pluripotent stem cells) from the blood cells of a DBA patient for the first time by reprogramming the early-stage erythroid progenitor cells that were obtained by culturing the peripheral blood mono-nucleated cells from 5 ml of blood, in an erythroid culture medium for seven days. We could isolate 23 DBA iPSC colonies, out of which only two colonies (DBA-C10 and DBA-C11) could be cultured for >30 passages, whereas the rest differentiated before 5 passages. The fibroblasts from the same patient also showed low reprogramming efficiency compared to the control, consistent with the earlier reports. Hematopoietic induction from blood-derived DBA iPSC clones was found defective in our study. We used a set of 5 stem cell factors (ERG, RORA, HOXA9, MYB, SOX4) in iPSCs, to restore the hematopoietic defect. These 5 stem cell transcription factors (5TFs) were reported originally to expand iPSC derived HSPCs (hematopoietic stem and progenitor cells). For the efficient generation of HSPCs, we generated iPSC lines with inducible 5TFs to induce their expression only during hematopoietic differentiation. The defect in hematopoietic differentiation of DBA iPSCs was successfully restored by the expression of 5TF. The HSPCs generated from 5TF DBA iPSCs could be expanded in serum-free condition. In an alternative model, we aimed to generate isogenic RP mutant iPSCs by CRISPR-Cas9 and base editing methods. However, we observed that the abrupt disruption of ribosome biogenesis genes leads to lethality in iPSCs. Our results showed that RP expression is critical for reprogramming and maintenance of iPSCs and their hematopoietic differentiation. The attempts to generate heterozygous mutations by gene editing created an abrupt disruption of protein synthesis and affected the survival of iPSCs. These findings provided new insights into the iPSC-based disease modelling of DBA.

Funding Source: Department of BiotechnoloiPSCgy, Government of India and Department of Biotechnology-Wellcome Alliance
Keywords: iPSC, DBA, Disease-Modeling

TOPIC: CARDIAC

824

USING IPSC-DERIVED VASCULAR ORGANOIDS AS ANGIOGENESIS MODEL

Trillhaase, Anja - *Institute for Cardiogenetics, University of Luebeck, Germany*
Ziegler, Wilko - *Institute for Cardiogenetics, University of Luebeck, Germany*
Erdmann, Jeanette - *Institute for Cardiogenetics, University of Luebeck, Germany*
Aherrahrour, Zouhair - *Institute for Cardiogenetics, University of Luebeck, Germany*

Abstract: Coronary artery disease (CAD) is the major cause of deaths worldwide. Until today almost 300 CAD risk genes or loci have been identified in genome-wide association studies (GWASs) to be associated with CAD. Mirroring the complex disease, these risk genes are involved in different pathways or mechanisms contributing to the progression of the disease including lipid metabolism, blood pressure, mitosis & proliferation, neovascularization or neoangiogenesis, NO-signaling, vascular remodeling, gene regulation, or inflammation. Human induced pluripotent stem cells (iPSCs) offer a great model as they overcome various problems that occur with primary cells. Stem cell derived 3D models can be generated, allowing a more physiological situation that can be analyzed in vitro. Vascular organoids have first been described in 2019 by Reiner Wimmer and colleagues derived from iPSCs as well as embryonic stem cells. The described vascular organ-

oids were made up of endothelial cells (ECs) as well as pericytes (PCs), connected to the blood system of mice after implantation and were perfused. We use these vascular organoids to develop a new stem cell-derived angiogenesis assay. Neovascularization, or angiogenesis, is a common feature in atherosclerosis and leads to disease progression. After embedding vascular aggregates into a collagen/matrigel matrix we either stimulate or inhibit the growth of vessel sprouts using different substances like VEGF-A or itraconazole respectively. Here, we demonstrate, that the treatment with stimulators such as VEGF-a enhances vessel sprouting, while inhibition with for example itraconazole blocks it. Therefore, this model can be used to study the effect CAD risk genes on angiogenesis in a 3D in vitro system.

Keywords: vascular organoids, angiogenesis model, human iPSCs

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EXTRACELLULAR MATRIX ENVIRONMENT AND MUSCLE CONTRACTION IS REGULATED BY VASCULARIZATION OF HUMAN CARDIAC ORGANOIDS

Voges, Holly K. - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Parker, Benjamin - *Anatomy and Physiology, The University of Melbourne, Australia*
Quaife-Ryan, Gregory - *Chronic Disorders, QIMR Berghofer, Brisbane, Australia*
Del Monte-Nieto, Gonzalo - *Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia*
Elliott, David - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Palpant, Nathan - *Centre for Cardiac and Vascular Biology, Institute for Molecular Bioscience, Brisbane, Australia*
Mills, Richard - *Chronic Disorders, QIMR Berghofer, Brisbane, Australia*
Porrello, Enzo - *Murdoch Children's Research Institute, Cell Biology, Melbourne, Australia*
Hudson, James - *Chronic Disorders, QIMR Berghofer, Brisbane, Australia*

Abstract: Multicellular composition of the heart is important for regulating organ development and responses to injury, however the role of cardiac cell types in organ maturation is not well characterised. In this study we characterised a vascularised cardiac organoid model (vhCO) derived from human pluripotent stem cells to better understand cell-cell interactions in human heart tissue. We show that endothelial cells in vhCO increase organoid force of contraction and enhance the expression of mature sarcomeric proteins and extracellular matrix (ECM) components. Through proteomics, we identified LAMA5 as an important component of the ECM network that enhances force of contraction. Subsequent knockdown of LAMA5 specifically in endothelial cells down regulate force of contraction and reverts the ECM protein profile. We also show that endothelial cells are important for modelling cardiac dysfunction induced by cytokine storm following COVID-19 infection. This study identifies matrix regulatory functions of endothelial cells that govern cardiac maturation and also highlights the importance of organoid multicellularity for disease modelling.

Keywords: cardiac organoids, endothelial cells, extracellular matrix

TOPIC: EARLY EMBRYO

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SOXE FACTORS CONTROL FLUID HOMEOSTASIS IN THE INNER EAR FOR HEARING REVEALED IN A MOUSE MODEL OF THE CAMPOMELIC DYSPLASIA SOX9Y440X MUTATION

Cheah, Kathryn S. - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Szeto, Irene - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Chu, Ka Chi - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Chen, Pekai - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Chu, Daniel - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Au, Tiffany - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Leung, keith - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Huang, Yong Heng – *Genome Regulation Laboratory, Guangzhou Institutes of Biomedicine and Health, Guangzhou, China*
Chan, Ying Shing – *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Jauch, Ralf - *School of Biomedical Sciences, University of Hong Kong, Hong Kong, Hong Kong*
Chan, Wood Yee - *School of Biomedical Sciences, Chinese University of Hong Kong*
Fritzsch, Bernd - *Department of Biology, University of Iowa, Iowa City, IA, USA*
Sham, Mai Har - *School of Biomedical Sciences, University of Hong Kong*
Lovell-Badge, Robin - *Stem Cells, Francis Crick Institute, London, UK*

Abstract: The in vivo mechanisms underlying dominant syndromes caused by mutations in SOXE (SOX9, SOX10) transcription factors, either when they are expressed alone or are co-expressed, are ill-defined. We created a mouse model for the campomelic dysplasia SOX9Y440X mutation which truncates the transactivation domain but leaves DNA binding and dimerization intact. Here, we find SOX9Y440X causes deafness via distinct mechanisms in the endolymphatic sac (ES)/duct and cochlea. By contrast, conditional heterozygous Sox9 null mice are normal. During the ES development of Sox9Y440X/+ heterozygotes, genes important for ionic homeostasis and Sox10 expression are downregulated and there is developmental persistence of progenitors resulting in fewer mature cells. Sox10 heterozygous null mutants also display persistence of ES/duct progenitors. By contrast, SOX10 retains its expression in the early Sox9Y440X/+ mutant cochlea, and later, in the developing stria vascularis. Dominant interference by SOX9Y440X impairs the cooperative activity of SOX9-SOX10 heterodimers, which normally repress expression of the water channel Aquaporin-3 in the cochlea. Our study shows that for a functioning endolymphatic system in the inner ear, SOX9 regulates Sox10 and, depending on cell-type and target gene, it can either work independently of, or cooperatively with, SOX10. SOX9Y440X can interfere with the activity of both SOXE factors, exerting effects that can be classified as hypomorphic or dominant negative. This model of disruption of transcription factor partnerships may be applicable to congeni-

tal deafness, which affects approximately 0.3% of newborns, and other syndromic disorders.

Funding Source: Hong Kong HKU7222/97M, HKU2/02C, HKU4/05C, AoE/M-04/04, T12-708/12N and the Jimmy and Emily Tang Professorship.
Keywords: SOXE control of inner ear development., Disease mechanisms, Transcriptional regulation

TOPIC: EPITHELIAL_LUNG

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FABRICATION OF AN BRONCHIAL EPITHELIUM MODEL INNERVATED BY SENSORY NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS IPSC

Foisset, Florent - *34295, IRMB U1183, Montpellier, France*
Lehalle, Christine - *UMR 7200 -LIT, CNRS et Université de Strasbourg, Faculté de Pharmacie, Universite De Strasbourg, Illkirch, France*
Nasri, Amel - *U1183- IRMB, INSERM, Université de Montpellier, France*
Vachier, Isabelle - *Département des Maladies Respiratoires, CHU de Montpellier, France*
Assou, Said - *U1183- IRMB, INSERM, Université de Montpellier, France*
Bourdin, Arnaud - *Département des Maladies Respiratoires, CHU de Montpellier, France*
Frossard, Nelly - *UMR 7200 -LIT, CNRS et Université de Strasbourg, Faculté de Pharmacie, Université de Strasbourg, Illkirch, France*
De Vos, John - *U1183- IRMB, INSERM, Université de Montpellier, France*

Abstract: An innervated bronchial epithelium model will be a good model to help understanding respiratory pathologies such as COPD (third leading cause of death in the world) and severe asthma for which current treatments are still ineffective. In fact, such a model differentiated from reprogrammed circulating cells of patients into iPSCs will allow the screening and pharmacological studies for ultra-personalized medicine. The project involves the construction of a subepithelial mucosa. For this purpose, an extra cellular matrix made in the laboratory composed of a mixture of collagen and chitosan is infiltrated by human bronchial fibroblasts and iPSC-derived sensory neurons are seeded on one side of the matrix. iPSC differentiated into functional bronchial epithelium will be added on the other side, using our previously published protocol. We obtained a submucosa with a thickened extracellular matrix, and the manufacturing of a basal lamina. An iPSC-derived bronchial epithelium after 40 days of differentiation was obtained on the top of this lamina. HES staining on paraffin section shows the presence of a pseudo stratified epithelium with different cell types including ciliated cells, confirmed by immunostaining. Regarding the innervation, iPSCs-derived sensory neurons were found by immunofluorescence, however there is no complete colonization. We successfully obtained a human iPSC-derived bronchial epithelium leading of a sub-epithelium mucosa. Developments are underway to improve the innervation. Finally, functional tests are also underway to see the impact of innervation on our epithelium.

Keywords: HUMAN INDUCED PLURIPOTENT STEM CELLS, BRONCHIAL EPITHELIUM, SENSORY NEURONS

TOPIC: EYE AND RETINA

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CHARACTERISATION OF STARGARDT'S IPSC-DERIVED RETINAL ORGANOIDS

Mangala, Melissa M. - *Stem Cell Medicine Group, Children's Medical Research Institute, Westmead, Australia*
Lin, Benjamin - *Stem Cell Medicine, Children's Medical Research Institute, Westmead, Australia*
Wong, Emilie - *Stem Cell Medicine, Children's Medical Research Institute, Westmead, Australia*
Aryamanesh, Nader - *Bioinformatics, Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Gonzalez-Cordero, Anai - *Stem Cell Medicine, Children's Medical Research Institute, Westmead, Australia*

Abstract: Stargardt's disease (STG1) is one of the most common inherited retinal diseases, with no current treatment. STG1 is characterised by loss of light-sensing photoreceptor cells in the macular region of the retina, resulting in irreversible vision loss and blindness. STG1 is caused by mutations in the ATP-binding cassette transporter gene, ABCA4, which is highly expressed in photoreceptor cells. Here, we modelled retinal organoids derived from induced pluripotent stem cells (iPSCs) to recapitulate the development of the human retina. In this study we generated two iPSC Stargardt's lines from patients, one with a severe form of STG1 and the other a mild form of STG1 known as foveal sparring. Using a well-established 2D/3D retinal differentiation protocol we generated STG1 iPSC-derived organoids containing all retinal cell types. Similar to control organoids and human adult retina these diseased organoids developed normal-looking photoreceptor cells expressing mature photoreceptor markers. Furthermore, we showed that STG1 organoids expressed different levels of ABCA4 protein compared to controls. In both patients the ABCA4 mutations are present in intron-exon boundaries and are predicted to cause splicing defects, this biomarker of disease was confirmed by RT-PCR on retinal organoids. Finally, single cell RNA sequencing demonstrated differences in the proportion of photoreceptor cells and the presence of differentially expressed genes, which will be crucial to further elucidate disease pathophysiology. Together, our data provides insights into Stargardt's disease characteristics and mechanisms. Development of iPSC-derived retinal organoids will provide a robust screening model to demonstrate efficacy for gene therapy strategies to treat Stargardt's disease.

Keywords: Retinal Organoids, Stargardt's, Disease model

TOPIC: KIDNEY

834

NOVEL PODOCIN MUTANTS MISTRAFFICKING IN IPSC- DERIVED KIDNEY ORGANOID MODELS OF CONGENITAL NEPHROTIC SYNDROME

Dorison, Aude - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Irene, Ghobrial - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Howden, Sara - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Graham, Alison - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*

Peiris, Thanushi - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Das, Mithun - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
See, Michael - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Forbes, Thomas - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Lawlor, Kynan - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Ramialison, Mirana - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*
Little, Melissa - *Kidney Regeneration, Murdoch Childrens Research Institute, Parkville, Australia*

Abstract: Nephrotic syndrome (NS) is one of the most common monogenic kidney diseases with NPHS2 mutations found in most children >1 month of age. Missense NPHS2 mutations result in mistrafficking of the encoded transmembrane podocyte specific protein, Podocin. Such studies overexpressed mutant proteins in immortalised 2D cultures, which may not reflect the in vivo consequences of the mutation. We generated NPHS2 mutant iPSC-derived kidney organoids as a model to dissect the pathogenic process of NS. We have simultaneously reprogrammed and CRISPR/Cas9 gene edited a control human fibroblast line, generating a control wild type (WT) line and 5 iPSC lines containing mutations of the endogenous NPHS2 locus leading to the protein changes: G92C, P118L, R138Q, R168H and R291W. All lines were differentiated into kidney organoids following our published directed differentiation protocol. Podocin localisation in subcellular compartments was assessed by immunofluorescence and the transcriptomic profile of glomeruli isolated from kidney organoids was analysed by bulk RNA sequencing. All mutant lines revealed an evident but reduced expression of the Podocin protein as shown by immunostaining and western blotting. Podocin mutant and WT proteins were detected in various cell compartments involved in protein trafficking with mutants accumulating in specific organelles. While G92C accumulated mainly at the membrane, P118L and R138Q were retained in the endoplasmic reticulum (ER) as previously described. Co-staining with GM130 demonstrated a partial Golgi retention for P118L, R168H and R291W. Finally, R291W was found to partially accumulate in late endosomes. Transcriptomic analysis of healthy and mutant kidney organoid-derived glomeruli showed a significant decrease of NPHS2 mRNA expression in G92C. Gene ontology enrichment analysis indicated the modulation of cell adhesion genes in all mutants as well as presence of an oxidative stress signature in P118L mutant podocytes. Discrepancies between previous reports and our findings highlight the need for a more appropriate model to study the pathobiology of NPHS2 mutations. This work will allow us to explore approaches to rescue individual Podocin defects guiding the development of new therapeutic strategies.

Keywords: Kidney organoids, Congenital nephrotic syndrome, Disease modelling

TOPIC: LIVER

836

PATIENT-SPECIFIC IPSCS FOR HEMOPHILIA B DISEASE MODELING AND RECOVERY OF FIX CLOTTING ACTIVITY AFTER CRISPR/CAS9 MODIFICATION

Luce, Eléanor - *UMR_S1193, Inserm, Villejuif, France*
Steichen, Clara - *UMR_S1193, INSERM, Villejuif, France*
Allouche, Mickael - *UMR_S1193, INSERM, Villejuif, France*
Messina, Antonietta - *UMR_S1193, INSERM, Villejuif, France*
Heslan, Jean Marie - *UMR_S1064, INSERM, Nantes, France*
Lambert, Thierry - *Centre de Référence pour le Traitement des Hémophiles, Hôpital de Bicêtre, Kremlin Bicetre, France*
Weber, Anne - *UMR_S1193, INSERM, Villejuif, France*
Nguyen, Tuan Huy - *UMR_S1064, INSERM, Nantes, France*
Christophe, Olivier - *UMR_S1176, INSERM, Kremlin Bicetre, France*
Dubart-Kupperschmitt, Anne - *UMR_S1193, INSERM, Villejuif, France*

Abstract: Hemophilia B (HB) is a monogenic disease characterized by a reduced activity of circulating clotting factor IX (FIX), synthesized by the hepatocytes. Current treatment based on regular intravenous injections of FIX is very restrictive, costly and only palliative. Gene therapy trials show promising results but not all patients are eligible and their long-term efficacy is still unknown. It is thus important to explore other strategies. To demonstrate the feasibility of a personalized gene/cell therapy approach, we reprogrammed skin fibroblasts from a severe hemophilia B patient (FIX activity < 1%) into induced pluripotent stem cells (hiPSCs). We used CRISPR/Cas9 technology to target the genomic insertion of an F9 mini-gene at the AAVS1 safe harbor locus. Non-corrected and corrected hiPSCs were differentiated into hepatocytes in both 2D and 3D culture systems. Differentiated cells expressed hepatocyte-specific markers such as HNF4α, HNF1α, α1AT, ALB and ATP7B. They exhibited signs of epithelial polarity, as shown by the lateral membrane staining of ZO-1, occludin and claudin-1. Gene and protein analyses highlighted improved maturation of the cells differentiated in 3D versus 2D, as reflected by the disappearance of AFP and the fetal cytochrome P450 3A7 (CYP3A7) in 3D spheroids, as well as the expression of mature hepatocyte markers such as the CYP3A4 and CYP2B6, the high density lipoprotein receptor (scavenger receptor B1), the cytokeratin 8, the connexin-32 and the bilirubin (Bil-UGT). 3D-differentiated cells acquired complex polarization highlighted by the expression of MDR3 at the biliary pole of hepatocytes as well as the bile salt export pump. In vitro studies showed that FIX was produced by the differentiated cells in both 2D and 3D culture systems. However, only the 3D system led to fully mature hepatocytes and thus permitted us to detect the correctly post-translationally modified FIX with in vitro clotting activity. Finally, we assessed the in vivo therapeutic efficacy of this approach in a mouse model of HB. PCR and immunohistochemistry analyses indicated a good engraftment of hiPSC-derived hepatic cells as shown by the expression of hCYP3A4, hHNF1a, hBil-UGT, hOATP1B3, hALB and hBSEP. Finally, the FIX activity detected in the plasma of transplanted animals confirmed the rescue of the phenotype.

Keywords: Hemophilia B, disease modeling, spheroids

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NEURAL STEM CELLS IN HUMAN CEREBRAL ORGANOIDS EXPRESS GLYCOEPITOPES IN DISTINCT PATTERNS

Roll, Lars - *Department of Cell Morphology and Molecular Neurobiology, Ruhr University Bochum, Germany*
Lessmann, Katrin - *Department of Cell Morphology and Molecular Neurobiology, Ruhr University Bochum, Germany*
Berg, Michael - *Department of Cell Morphology and Molecular Neurobiology, Ruhr University Bochum, Germany*
Brüstle, Oliver - *Institute of Reconstructive Neurobiology, University of Bonn Medical Faculty & University Hospital Bonn, Germany*
Faissner, Andreas - *Department of Cell Morphology and Molecular Neurobiology, Ruhr University Bochum, Germany*

Abstract: Regeneration of the human central nervous system is limited, which raises the question whether mechanisms found in the developing organism might be re-activated to support functional recovery. In this context, detailed knowledge of signals that control proliferation, differentiation, migration, axon growth and other processes is of interest. The extracellular matrix (ECM) with its numerous molecules provides such signals. Glycosylation is a common modification of ECM molecules. Here, sugars are attached to a core protein, thereby influence its interaction with other molecules, which in the end can affect the cell fate. Defined carbohydrate motifs have been shown to be associated with neural stem/progenitor cells. In our study, we assess the expression profiles of LewisX (LeX) and human natural killer-1 (HNK-1) trisaccharide motifs and of the dermatan sulfate-dependent-1 (DSD-1) chondroitin sulfate epitope during human development. We derived cerebral organoids from human induced pluripotent stem cells (hiPSCs) as three-dimensional model for early stages of the nervous system and used the following monoclonal antibodies (mAbs) for immunohistochemical analysis: mAb 487LeX, which binds LeX motifs at terminal positions in the carbohydrates, mAb 5750LeX, which binds internal repeats of LeX, mAb HNK-1 412 for the HNK-1 motif and mAb 473HD for the DSD-1 epitope. The analysis revealed individual expression patterns of the carbohydrate motifs that differed with regard to rosette-shaped structures, formed by neural stem cells. They resemble the neural tube in vitro. The 487LeX and HNK-1 epitopes were detected on the cell surfaces of these rosettes, in contrast to the 5750LeX signal, which was found most prominent in the lumen, and the DSD-1 epitope, which was enriched at the outer border. Via RT-PCR, we verified the expression of the carbohydrate sulfotransferases CHST3, CHST7, CHST11, CHST12, CHST14 and UST as well as of the fucosyltransferases FUT4 and FUT9 on mRNA level. These enzymes are involved in and therefore essential for the synthesis of the glycoepitopes. The specific expression patterns observed in human cerebral organoids indicate very distinct functions of these ECM motifs in the developing human central nervous system. This knowledge might be exploited for therapeutic approaches in the future.

Funding Source: Support from German Research Foundation (DFG, SPP 1757, FA 159/20-1,2, Fa 159/23-1) to AF.
Keywords: cerebral organoid, neural stem cell, glycoepitope

840

HUMAN IPS CELL-DERIVED NEURAL PRECURSORS WITH REGIONAL IDENTITY GIVE RISE TO MATURE AND FUNCTIONAL GLIAL CELLS

Motono, Makoto - *Department of Anatomy and Cell Biology, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
Kato, Hidemasa - *Department of Developmental Biology and Functional Genomics, Ehime University, Toon, Japan*
Kondo, Yoichi - *Department of Anatomy and Cell Biology, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*

Abstract: Recent single-cell RNA sequence studies show that astrocytes are morphologically and functionally diverse depending on the brain regions and play critical roles in neurodevelopmental diseases such as Rett syndrome. Alexander's disease, another astrocyte disease, is caused by a point mutation in the GFAP gene and affects different brain regions at different timings depending on the site of mutations. In this study, to understand the diversification of astrocytes, we sought to induce astrocytes from regionally specified neural precursors from human iPS cells and investigate differences among them. First, to differentiate neural precursors compatible with various brain regions' identity, we carefully reviewed previous induction methods, mainly reaction timing and strength of morphogens involved in anterior-posterior and dorsal-ventral axis, and sought to modify them to faithfully follow developmental trajectories. Using the improved rationale-based differentiation protocols, we obtained neural precursors of various regions of the central nervous system in unprecedentedly high efficiencies: forebrain (cerebral cortex and medial ganglionic eminence), ventral midbrain, and spinal cord. Next, we sought to differentiate glial progenitor cells from these various neural precursors and were able to independently induce mature astrocytes after a prolonged cell culture period (more than five months). In the future, we plan to purify the induced glial cells and investigate their characteristics to reveal the regional specificity of glial cells. Furthermore, we plan to co-culture these glial cells and the matched neuronal precursors or neurons for drug discovery and toxicity study.

Keywords: Astrocyte differentiation, central nervous system, glial progenitor

842

ENHANCED MITOCHONDRIAL BIOGENESIS REFLECTS SCHIZOPHRENIA STATUS IN INDIVIDUALS WITH 22Q11.2 DELETION SYNDROME

Li, Jianping - *Psychiatry, CHOP, Philadelphia, PA, USA*
Anderson, Stewart - *Psychiatry, Children's Hospital of Philadelphia, PA, USA*

Abstract: Previously we found compensatory upregulation of mitochondrial biogenesis in 22qDS patients may reduce SZ risk. Therefore, strategies that improve mitochondrial biogenesis in neurons may be therapeutic in 22qDS-related SZ. compelling targets for the enhancement of mitochondrial biogenesis are the evolutionarily-conserved microRNAs, miR-181a/b, which inhibit translation of PGC1α and other key proteins necessary for mitochondrial function. As a result, knockdown of miR-181a/b improves mitochondrial function, neurite complexity, and synaptic activity in a variety of brain cell types. We find that expression of

a miR181 antagomir rescues the deficit of mitochondrial biogenesis seen in IPSC-derived neurons from 22qDS with SZ. Furthermore, knockdown of miR-181a prior to brain injury is sufficient to promote the survival of parvalbumin-expressing (PV) GABAergic interneurons that drive normal neuronal inhibitory signaling and are often dysregulated in SZ. Since chemogenetic activation of ventral hippocampal CA1 (vCA1) PV interneurons rescues behavioral/synaptic phenotypes in a mouse model of 22qDS, inhibition of miR-181a/b may also be therapeutic toward 22qDS by facilitating mitochondrial biogenesis and GABAergic activity. We will evaluate whether mitochondrial biogenesis represents a feasible target for SZ related phenotypes in patients with 22qDS, which ultimately be applied to other disorders associated with mitochondrial dysfunction.

Keywords: 22qDS-related schizophrenia, mitochondrial biogenesis, miR-181a/b

844

DISSECTING AXONAL DEGENERATION OF HUMAN CORTICAL NEURONS USING PLURIPOTENT STEM CELLS MODELS OF HEREDITARY SPASTIC PARAPLEGIA TYPE 11

Chen, Zhenyu - *College of Medicine, University of Illinois, Rockford, IL, USA*
Chai, Eric - *School of Medicine, University of Illinois, Rockford, IL, USA*
Mou, Yongchao - *School of Medicine, University of Illinois, Rockford, IL, USA*
Roda, Ricardo - *National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA*
Blackstone, Craig - *Department of Neurology, Massachusetts General Hospital, Boston, MA, USA*
Li, Xue-jun - *School of Medicine, University of Illinois, Rockford, IL, USA*

Abstract: Hereditary spastic paraplegia (HSP) is a heterogeneous group of inherited diseases characterized by axonal degeneration of corticospinal motor neurons, leading to spasticity and weakness of the leg and hip muscles. SPG11, a common recessive form of HSP, is caused by mutations in the SPATACSIN (SPG11) gene. Though mitochondrial dysfunction has been observed in HSP neurons, how human axons degenerate in HSPs and whether targeting mitochondrial dysfunction can rescue disease phenotypes remain largely unclear. In this study, we examined the role of mitochondria in the degeneration of cortical neuron axons in SPG11. In addition to patient-specific induced pluripotent stem cell (iPSC) model, we also knocked-in disease-specific mutation of SPATACSIN into human embryonic stem cells (hESCs) using CRISPR-cas9-mediated gene editing. Cortical projection neurons (PNs) derived from both SPG11 patient iPSC and SPG11 mutation knock-in hESC exhibited impaired axonal transport of mitochondria, accumulated neurite swellings, and increased disease-related phospho-neurofilament release, which recapitulate disease-specific axonal degeneration. Moreover, these SPG11 cortical neurons exhibited reduced mitochondrial length and area, aberrant mitochondrial membrane potential and ATP production, as well as the accumulation of abnormal neurofilament aggregations, implying mitochondrial dysfunction and cytoskeleton disorganization in axonal defects of SPG11 neurons. Notably, treatment of a short peptide that inhibits mitochondrial fission mitigated mitochondrial dysfunction and abnormal neurofilament aggregations, leading to the rescue of axonal defects of SPG11 cortical neurons. Finally, restoring the SPG11 levels by lentiviral expression of wild-type SPATACSIN rescued the mitochondrial and axonal defects, confirming the cause-effect relationship between loss of SPG11 function and disease phenotypes. Taken together, our data demonstrate that mitochondrial defects underlie cytoskeleton disorganization and axonal degeneration of HSP neurons, and highlight the importance of targeting these pathologies to develop therapies for HSP.

Funding Source: This work is supported by the Spastic Paraplegia Foundation, National Institutes of Health, and the Blazer Foundation.
Keywords: Hereditary spastic paraplegia, Human pluripotent stem cells, Neural degeneration

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CHARACTERIZING THE RELATIONSHIP OF LGL1 AND AKT IN MIGRATION OF MURINE NEURAL PROGENITOR CELLS

Pedicino, Natalie R. - *Biological Sciences, Humboldt State University, Santa Cruz, CA, USA*
Tzeciak, Jacqueline - *Biological Sciences, Humboldt State University, Arcata, CA, USA*
King, Megan - *Biological Sciences, Humboldt State University, Humboldt, CA, USA*
Light, Brandon - *Biological Sciences, Humboldt State University, Arcata, CA, USA*
Otis, Sharon - *Biological Sciences, Humboldt State University, Arcata, CA, USA*
LaCourse, Monique - *Biological Sciences, Humboldt State University, Arcata, CA, USA*
Collins, Hannah - *Biological Sciences, Humboldt State University, Arcata, CA, USA*
Petritsch, Claudia - *Neurosurgery, Stanford University, Palo Alto, CA, USA*
Sprowles, Amy - *Biological Sciences, Humboldt State University, Arcata, CA, USA*

Abstract: Asymmetric cell division and migration are critical for neural stem cell differentiation and brain development. When these processes are dysregulated in neural progenitor cells (NPCs), developmental defects and diseases like glioblastoma multiforme (GBM) can result. Lgl1 is a tumor suppressor gene first characterized in Drosophila neuroblasts where it was shown to regulate apical-basal cellular polarity through its association with the Par complex. The PI3K/AKT signaling cascade is also regulated by Par signaling and involved in cellular migration. To investigate the role of LGL1 on the regulation of NPC migration, NPCs were cultured from genetically matched Lgl1-/- and Lgl1+/+ primary cell lines isolated from the subventricular zone (SVZ) and corpus collosum (CC) of P30 mice. Spheroid and scratch test migration assays revealed that loss of Lgl1 increased migration rates by up to two-fold, demonstrating that LGL1 regulates NPC migration. To see if loss of Lgl1 could be influencing migration through PI3K/AKT signaling, we used targeted drugs to inhibit mTOR and PI3K in the same cellular migration assays and evaluated the phosphorylation states of AKT. Our findings suggest that changes in Akt phosphorylation through PDK/PI3K sites are a key link between LGL1 and the migratory defects we observed. Our results are also consistent with findings that loss of Lgl1 affect membrane dynamics and increase rates of cellular migration. Our results contribute to the understanding how loss of cell polarity

affects neural cell differentiation, cancer cell properties, and GBM progression.

Keywords: NPC, LGL1, Migration

848

16P11.2 DELETION DISRUPTS CORTICAL DEVELOPMENT AND METABOLIC CONTENT IN PATIENT-SPECIFIC HUMAN MODELS OF DISEASE

Sundberg, Maria K. - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Polanco, Taryn - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Bajic, Aleksandar - *Neurobiology, Baylor College of Medicine, Houston, TX, USA*
Osenberg, Sivan - *Neurobiology, Baylor College of Medicine, Houston, TX, USA*
Cappuccio, Gerarda - *Neurobiology, Baylor College of Medicine, Houston, TX, USA*
Maletic-Savatic, Mirjana - *Neurobiology, Baylor College of Medicine, Houston, TX, USA*
Sahin, Mustafa - *Neurology, Boston Children's Hospital, Boston, MA, USA*

Abstract: Rare genetic copy number variations of 16p11.2 region cause a variety of different neurodevelopmental disorders, including autism spectrum disorder, neuropsychiatric disorders, deficits in speech development and deficits in social communication. 16p11.2 deletion has also been associated with increased head circumference and body mass index. The molecular and cellular mechanisms underlying these disorders remain unresolved and current treatment are limited or inefficient. Thus, better understanding of the disease mechanisms is critical to develop more efficient therapies in the future. Previously, we have characterized the disease phenotypes of dopaminergic (DA) neurons derived from CRISPR-Cas9 edited iPSCs with 16p11.2 deletions or 16p11.2 duplications. We discovered that DA neurons with 16p11.2 deletion had increased synaptic marker expression and hyperactive networks compared to healthy control DA neurons. We also observed increased RhoA expression in the 16p11.2 deletion DA neurons. Treatment with Rhosin, a specific RhoA pathway inhibitor, rescued the abnormal morphology of DA neurons and the hyperactivity of the 16p11.2 deletion neuron networks. To study these pathways further, here we have asked how 16p11.2 deletion affects cortical development in these disorders. To answer this question, we used different molecular biology methods and neuronal differentiation protocols, including two-dimensional transcription factor guided cortical neuron differentiations, three-dimensional forebrain organoids, RNA sequencing, metabolomics and functional assays with micro electrode array platforms. Our results suggest that the 16p11.2 deletion disturbs cortical development substantially, unveiling specific molecular pathways that may be targeted to treat these deficits in cell culture models.

Keywords: Neurodevelopmental disorders, Neuropsychiatric disorders, Cortical neurons

TOPIC: PANCREAS

850

GENERATION OF FUNCTIONAL PANCREATIC B CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS WITH PHYSIOLOGICAL CELL CULTURE MEDIUM

Kato, Yusuke - *Department of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan*
Shiraki, Nobuaki - *Department of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan*
Kume, Shoen - *Department of Life Science and Technology, Tokyo Institute of Technology, Yokohama, Japan*

Abstract: Amino acids and other nutrients in the culture medium are increasingly important in supporting the maturation of pluripotent stem cell-derived pancreatic β (SC-β) cells. In vivo experiments, pancreatic beta cells have been suggested to sense postnatal nutritional changes through the mTORC1 pathway and converse from amino acid-responsive to glucose-responsive insulin secretion (GSIS)(Helman A. et al., 2020). However, to date, SC-β cells have been generated using culture media originally developed for maintaining xenogeneic cell lines or cancer cells. These media do not mimic normal blood nutrient concentrations. For example, MCDB131 and DMEM are media commonly used in pancreatic differentiation, containing higher branched-chain amino acids and vitamins than human blood. We aim to generate functional human induced pluripotent stem cells-derived pancreatic β (iPS-β) cells, utilizing a physiological cell culture medium to elucidate the advantage of this medium over a conventional medium. We combined a novel six-step rotating culture system with Human Plasma Like Medium (HPLM). The differentiated cells in each step were collected and assayed for protein expression levels and GSIS to examine their functionality. We successfully generated and maintained functional iPS-β cells using HPLM as the basal media. In addition, the culture media were collected at each differentiation stage, and metabolites were analyzed using liquid chromatography-tandem mass spectrometry (LC/MS/MS). Metabolite analysis revealed absorption and excretion of specific amino acids and other metabolites at each step. This novel culture system can be used as a tool for drug discovery of type 1 or type 2 diabetes and a model for the studies for the Developmental Origins of Health and Disease (DOHaD).

Keywords: iPS-β cells, Human plasma-like medium, Primary metabolic analysis

TOPIC: PLURIPOTENT STEM CELLS

852

CCR7 REGULATES EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION AND MOTILITY BY PROMOTING THE EPITHELIAL-TO-MESENCHYMAL TRANSITION

Yoon, Eun-Ja - *Developmental Biology, Washington University, St. Louis, MO, USA*
Beltcheva, Mariana - *Developmental Biology, Washington University in St. Louis, MO, USA*
Dong, Chen - *Developmental Biology, Washington University in St. Louis, MO, USA*
Karvas, Rowan - *Developmental Biology, Washington University*

in St. Louis, MO, USA
Ali, Syed - *Developmental Biology, Washington University in St. Louis, MO, USA*
Gontarz, Paul - *Developmental Biology, Washington University in St. Louis, MO, USA*
Dietmann, Sabine - *Developmental Biology, Washington University in St. Louis, MO, USA*
Theunissen, Thorold - *Developmental Biology, Washington University in St. Louis, MO, USA*
Solnica-Krezel, Lilianna - *Washington University in St. Louis, Developmental Biology, St. Louis, MO, USA*

Abstract: Chemokines play important roles during mammalian blastocyst implantation and placentation by modulating trophoblast differentiation and motility. In the human blastocyst, the chemokine G protein-coupled receptor CCR7 is expressed in polar trophectoderm, which mediates implantation of the blastocyst into the uterus and forms the placenta. CCR7 is also expressed in placental trophoblasts, including cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast (EVT). However, the function of CCR7 during trophoblast differentiation has not been elucidated thus far. Here, we investigated CCR7 function in human trophoblast differentiation in vitro. Primed H9 WT and CCR7 KO hESCs were converted to naïve cells, which were cultured in human trophoblast stem cell (hTSC) medium to promote differentiation into hTSCs and subsequently into EVTs. Interestingly, CCR7 mutant cells differentiated into hTSCs but showed a greatly reduced number of motile spindle-shaped mature EVTs compared to WT. During EVT differentiation, CCR7 mutant cells exhibited reduced HLA-G expression at the protein level by flow cytometry and immunofluorescence (IF) staining. In addition, we generated 3D stem cell-derived trophoblast organoids (SC-TOs) from WT and CCR7 KO hTSCs and performed single-cell RNA-seq analyses, which revealed a reduced proportion of EVTs in CCR7 KO organoids. Using RNA seq and qRT-PCR, we detected lower expression of several epithelial-to-mesenchymal-transition (EMT) markers, including MMP2 and SNAI1, in CCR7 KO EVTs. Accordingly, IF staining showed that CCR7 KO EVTs expressed reduced levels of cytoplasmic MMP2 and nuclear SNAI1. Furthermore, CCR7 KO EVTs maintained expression of the epithelial markers ZO-1 and E-cadherin, which were downregulated in WT EVTs. To further investigate the function of CCR7 in EMT and EVT cell motility, we performed a transwell cell migration assay and observed a reduced number of migrating cells in CCR7 KO compared to WT EVTs. Altogether, these lines of evidence strongly support a critical role for CCR7 in promoting EMT and cell motility, which are important to anchor the placenta and remodel maternal spiral artery in vivo.

Funding Source: Children Discovery Institute grant from the Washington University School of Medicine in St. Louis.
Keywords: Chemokine G protein-coupled receptor CCR7, Trophoblast, Epithelial-to-mesenchymal-transition

POSTER SESSION II: EVEN

4:00 PM – 5:00 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: NT- GENERAL

502

CORNING MATRIBOT BIOPRINTER PRINTED DOMES FOR ORGANOID DRUG TESTING

Mogen, Austin Blake - *Field Applications, Corning Life Sciences, Phoenix, AZ, USA*
Ann, Ferrie - *Applications, Corning, Kennebunk, NY, USA*
Sherman, Hilary - *Applications, Corning, Kennebunk, NY, USA*

Abstract: Patient-derived pancreatic organoids are capable of recapitulating tissue from the original tumor. This makes them an ideal model for generating biobanks, compound library testing and personalized cancer drug screening. However, most organoid assays require extracellular matrices such as Corning Matrigel® matrix for self-organization and differentiation which can be challenging due to the hydrogel's temperature sensitivity and viscosity. Manually dispensing such matrices in the small volumes required for screening can lead to inaccurate or inconsistent organoid cultures. To address these challenges, we have developed an automated dispensing protocol utilizing a bioprinter designed to dispense viscous and temperature-sensitive hydrogels such as Matrigel matrix. Data presented here demonstrates the ability to dispense the same number of organoids consistently and accurately in a single 5 µL droplet centered into each well of a 96 well microplate achieving CVs of less than 15%. Using the developed assay, we were able to assess organoid toxicity from several chemotherapeutics that are traditionally used for treating pancreatic cancer. This data demonstrates an automated way to screen for the best drug choice for an individual's pancreatic cancer.

Keywords: bioprinter, organoid, drug testing

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

504

GENERATING A PERFUSABLE VASCULARIZED BRAIN ORGANOID MODEL USING MICROFLUIDICS AND PHOTODEGRADABLE POLYMER SCAFFOLDS

LaMontagne, Erin E. - *Bioengineering, University of California, San Diego, CA, USA*
Macdougall, Laura - *Chemical & Biological Engineering, University of Colorado Boulder, CO, USA*
Papes, Fabio - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Mayner, Jaimie - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Teng, Evan - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Sutherland, Bryan - *Chemical & Biomolecular Engineering, University of Delaware, Newark, DE, USA*
Anseth, Kristi - *Chemical & Biological Engineering, University of*

Colorado Boulder, CO, USA
Kloxin, April - *Chemical & Biomolecular Engineering, University of Delaware, Newark, DE, USA*
Muotri, Alysson - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Engler, Adam - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*

Abstract: Although brain organoids have advanced our understanding of the human brain, their growth, cellular complexity, tissue organization, and functionality are limited by the absence of vasculature. Current organoid vascularization efforts rely on either: (1) the in situ formation of neo-vessels in endothelial co-cultures, or (2) microfluidics channels that flow adjacent to organoids. Neither produces vasculature that resembles native cerebral vessels. We hypothesize that a system in which patent vessels form throughout the organoid as it matures will enable organoid growth beyond the diffusion limit of oxygen and promote the formation of more complex cerebral structures. To fabricate this unique system, we first developed a microfluidic device composed of an organoid chamber that is gated by pillars to restrict organoid motility. The device also contains posts onto which a biocompatible photodegradable fiber scaffold is woven in a square lattice resembling the cerebral vascular network. Photodegradable fibers are formed by extruding and crosslinking a solution of two multi-arm polyethylene glycol (PEG) components which contain allyl sulfide to facilitate degradation via radical addition. Brain organoids that we have generated from pluripotent stem cells (PSCs) are to be placed within the woven fiber network when at the neuroprogenitor cell (NPC) stage so that they grow around fibers as they enlarge to ~1 mm. Upon radical addition, the polymer scaffold will solubilize and provide the framework for a continuous, hollow lumen that will be seeded with patient-matched induced pluripotent stem cell (iPSC)-derived endothelial cells to generate vasculature. This model will enable the study of cerebrovascular pathophysiology in neurological diseases, e.g., Cockayne Syndrome, which cause irregular brain vasculature and electrophysiology.

Funding Source: This work is supported by the National Science Foundation's Graduate Research Fellowship Program and the facilities at UC San Diego's Sanford Consortium and Nano3.
Keywords: tissue engineering, vascular modeling, microfluidics

TOPIC: HEMATOPOIETIC SYSTEM

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OPTIMIZATION OF BETA-GLOBIN LENTIVIRAL VECTORS FOR SICKLE CELL DISEASE USING ALPHA-GLOBIN 3'UTRS

Orr, Katherine - *Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Woodland Hills, CA, USA*
Hart, Kevyn - *Department of Human Genetics, University Of California, Los Angeles, CA, USA*
Hollis, Roger - *3 Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA, USA*
Williams, David - *Divison of Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Brendel, Christian - *Divison of Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Kohn, Donald - *Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*

Abstract: Sickle cell disease is a monogenic blood disorder caused by a E6V mutation of beta-globin resulting in a sickled-globin, HbS. Deoxygenated conditions cause HbS to form sickled shaped RBCs and consequently, poor oxygen transport. Allogeneic hematopoietic stem cell transplants (HSCTs) can be a cure, however, the majority of patients with SCD cannot benefit from this due to a lack of unaffected HLA-matched donors due to risks including graft rejection and graft versus host disease (GvHD). This study aims to utilize a lentiviral vector (LVV) UV END IVS2 with the incorporation of an anti-sickling ßAS3-globin for the use in autologous HSCTs. In house expression data demonstrates alpha2-globin 3'UTRs result in better alpha-globin expression compared to beta-globin 3'UTRs. We hypothesize that replacing the beta-globin 3'UTR with alpha2-globin 3'UTR will increase ßAS3 expression. To further optimize this vector to achieve higher ßAS3 expression, we developed two vectors containing either an alpha2-globin 3'UTR or alpha2-globin 3'UTR with a downstream enhancer region, both located within the ßAS3 cassette. Replacing the 3'UTR in current LVVs may be of consideration as we have shown that this results in a 1.5 fold increase of ßAS3 mRNA expression.

Funding Source: This research was made supported by the UCLA-CSUN CIRM Bridges program and discretionary funds from an Endowment from the UCLA Broad Stem Cell Research Center.
Keywords: gene therapy, sickle cell disease, lentiviral vectors

TOPIC: IMMUNE SYSTEM

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ALLOGENEIC HSC-ENGINEERED INVARIANT NATURAL KILLER T (INKT) CELLS FOR CANCER IMMUNOTHERAPY

Yang, Lili - *Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA, USA*
Li, Yan-Ruide - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Zhou, Yang - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Chen, Xianhui - *Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA, USA*
Crooks, Gay - *Medicine, University of California, Los Angeles (UCLA), CA, USA*
Huang, Jie - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Kim, Yu Jeong - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Kohn, Donald - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Larson, Sarah - *Internal Medicine, University of California, Los Angeles (UCLA), CA, USA*
Lee, Derek - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Li, Zhe - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Ma, Feiyang - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Montel-Hagen, Amelie - *Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), CA, USA*

Pellegrini, Matteo - *Molecular, Cell and Developmental Biology, University of California, Los Angeles (UCLA), CA, USA*
Ribas, Antoni - *Molecular and Medical Pharmacology, University of California, Los Angeles (UCLA), CA, USA*
Sasine, Joshua - *Pediatrics, University of California, Los Angeles (UCLA), CA, USA*
Seet, Christopher - *Medicine, University of California, Los Angeles (UCLA), CA, USA*
Wang, Pin - *Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA, USA*
Wang, Xi - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Wang, Xiaoyan - *Medicine, University of California, Los Angeles (UCLA), CA, USA*
Wang, Yu-chen - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Witte, Owen - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Yu, Jiaji - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Zeng, Samuel - *MIMG, University of California, Los Angeles (UCLA), CA, USA*
Zhu, Yanni - *MIMG, University of California, Los Angeles (UCLA), CA, USA*

Abstract: Cell-based immunotherapy has become the new-generation cancer medicine, and “off-the-shelf” cell products that can be manufactured at large scale and distributed readily to treat patients are necessary. Invariant natural killer T (iNKT) cells are ideal cell carriers for developing allogeneic cell therapy because they are powerful immune cells targeting cancers without graft-versus-host disease (GvHD) risk. However, healthy donor blood contains extremely low numbers of endogenous iNKT cells. Here, by combining hematopoietic stem cell (HSC) gene engineering and in vitro differentiation, we generate human allogeneic HSC-engineered iNKT (AlloHSC-iNKT) cells at high yield and purity; these cells closely resemble endogenous iNKT cells, effectively target tumor cells using multiple mechanisms, and exhibit high safety and low immunogenicity. These cells can be further engineered with chimeric antigen receptor (CAR) to enhance tumor targeting or/and gene edited to ablate surface human leukocyte antigen (HLA) molecules and further reduce immunogenicity. Collectively, these preclinical studies demonstrate the feasibility and cancer therapy potential of AlloHSC-iNKT cell products and lay a foundation for their translational and clinical development.

Keywords: hematopoietic stem cell (HSC), invariant natural killer T (iNKT) cell, off-the-shelf cancer immunotherapy

TOPIC: MUSCULOSKELETAL

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BMP2 SURROGATE USING TUNABLE NANOBODIES

Takematsu, Eri - *Surgery, Stanford University, Palo Alto, CA, USA*
Zhao, Liming - *Surgery, Stanford University, Stanford, CA, USA*
Wang, Sicong - *Institute of Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Chan, Charles - *Surgery, Stanford University, Palo Alto, CA, USA*

Abstract: Bone morphogenic protein 2 (BMP2) is a powerful protein with key roles in developmental biology and applications in regenerative medicine. The critical aspects of BMP2 signalling range from inducing mesoderm and guiding the formation of the central nerve system in early embryonic development to repairing adult bone and cartilage damage in regenerative medicine. BMP2 is clinically approved for applications in bone repair, and studies have shown that BMP2 could increase the rate of bone fracture healing in patients by up to 90%. Despite its clinical utility, recombinant BMP2 as a drug possesses several shortcomings. Currently available delivery methods for BMP2 does not provide sufficiently accurate or precise signaling strength that is desirable for complex systems such as regeneration or developmental models. Additionally, the persistence of BMP2 responsive mechanisms in many adult cell types increases the potential for off-target effects. For instance, BMP2 also signals to innate immune cells such as monocytes and macrophages, causing inflammation and additional release of pro-inflammatory cytokines, resulting in pain and additional bone/cartilage resorption at the injury site. To overcome these limitations, we are engineering a synthetic BMP2 surrogate whose activity can be precisely tuned and explicitly targeted to skeletal stem cells. Our BMP2 surrogate comprises two nanobodies (NBs) with a solid affinity for BMP2 receptors, bringing the two receptors to proximity to initiate downstream signaling. The geometry of the construct could be adjusted to tune their signaling strength, and overall activity can be turned on/off by a cleavable linker between BMPR recognizing domains. To generate the BMP2 surrogate, we first synthesized the extracellular domain of BMPR1a and BMPR2. A yeast nanobody library was used to identify candidate NB1 and NB2 with sufficient affinity. These candidates NB1 and NB2 are fused with a peptide domain to provide conformational control of targeted BMPR1a and BMPR2. Regions in the surrogate construct are selectively designed to be rapidly degraded by enzymes expressed by untargeted immune cells, thus limiting off-target activity. This presentation will describe additional details of the design process and the new therapeutic opportunities that our BMP2 surrogate provides.

Funding Source: Japan Society for the Promotion of Science
Keywords: BMP2 surrogate, Bispecific nanobody, synthetic protein

TOPIC: NEURAL

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MEAMAPPER: A VISUALIZATION TOOL FOR MULTI-ELECTRODE ARRAY (MEA) DATA

Wooten, Alexander - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*
Bang, Anne - *Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA*

Abstract: Multi-well MEAs enable higher throughput analyses of hiPSC-derived neuronal networks, facilitating experiments using pharmacological manipulations and other perturbagen treatments, as well as studies of multiple patient and control lines in parallel. MEA recordings are data-rich, and analyses generate large, multiparametric data sets that can be challenging to manage, especially for multi-well formats. To address this issue, we developed a Shiny application, MEAmapper, that automates statistical analyses, hierarchical clustering, and generation of heatmaps for data visualization. A variety of customization options are built-in to an intuitive, graphical user interface (GUI), where pre-processed recording data is uploaded. Users can select which metrics to be included in the analysis, as well as choose

between heatmap visualizations for corrected p-values, Z-scores, percent change, and raw values. To aid in detection of data signatures, users can also select hierarchical clustering methods to be applied, including k-means. Additional customization options include visualizations of single or combined timepoints, and categorical annotations, such as drug class or target. In summary, MEAmapper significantly reduces analysis time, provides a rapid multiparametric view into recorded activity, and enables cross-site comparison of MEA data.

Funding Source: NIMH NCRCRG (U19MH106434), USAF
Keywords: multi-electrode array (MEA), graphical user interface (GUI), neuronal network

TOPIC: NT - GENERAL

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THE EKKO AND EKKO SELECT: ACOUSTIC-BASED CLOSED AND AUTOMATED SYSTEMS FOR CELL AND GENE THERAPY CGMP MANUFACTURING

Mora, Maria T. - *Acoustics Research and Development, MilliporeSigma, Springfield, IL, USA*
Tostoes, Rui - *Research and Development, MilliporeSigma, West Springfield, MA, USA*
Lipkens, Bart - *Research and Development, MilliporeSigma, West Springfield, MA, USA*
Kumar, Krishna - *Research and Development, MilliporeSigma, West Springfield, MA, USA*
Ross-Johnsrud, Benjamin - *Commercial, MilliporeSigma, West Springfield, MA, USA*
Saloio, Jack - *Research and Development, MilliporeSigma, Springfield, MA, USA*
Scott, Robert - *Research and Development, MilliporeSigma, West Springfield, MA, USA*

Abstract: Acoustic Cell Processing is a unique acousto-fluidics platform technology for minimal manipulation of cells using ultrasonic waves. The platform has broad applications in the field of cell and gene therapy, e.g., cell concentration and washing, acoustic affinity cell selection and label-free cell selection. The acoustic radiation force exerted by the ultrasonic field on the suspended cells in combination with fluid drag forces and gravitational forces is used to manipulate the cells and perform a certain cell processing unit operation, e.g., separate, concentrate, wash or select. The technology is single-use, continuous, and can be scaled up, down or out. It therefore allows for a flexible and modular approach that can be customized to process a desired cell count, cell culture volume or cell concentration within a given required process time. The ekko™ captures cells in the acoustic standing wave, enabling the cells to be concentrated and washed inside the ekko™ consumable. This instrument and consumable work in a closed and automated fashion and can be applied to various cell types for numerous applications, such as T cells, MSC and iPSC suspensions to perform any of the concentrate and wash unit operations, including media exchange, cryoprotectant wash out from frozen apheresis products, 200-fold volume reduction and wash pre-electroporation or final harvest and cryoprotectant addition pre-cryopreservation. In this work, we demonstrate high cell recovery, impurity wash out and buffer exchange in different CAR T workflow steps, hMSC and hPSC single cell suspension processing using the ekko™. The ekko™ Select uses (non-paramagnetic) affinity beads for positive or negative cell selection. A multi-dimensional acoustic standing wave is then used to separate the affinity bead-cell complexes from the unbound cells, thereby completing the process of cell capture. In this work we show multiple donor T cell selection from apheresis products with a final T cell purity of 95% and better than 70% cell recovery in our closed and automated ekko™ Select system.

Keywords: Cell Processing, Affinity Cell Selection, Cell Therapy Manufacturing

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RED2FLPE, A VERSATILE MULTICOLOR SYSTEM FOR IN VIVO CONDITIONAL MOSAIC KNOCKOUT

Wu, Szu-Hsien - *Koo Lab, Insitute for Molecular Biotechnology (IMBA), Vienna, Austria*
Boese, Ayse - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Colozza, Gabriele - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Gert, Krista - *Pauli Lab, Institute of Molecular Pathology, Vienna, Austria*
Hallay, Natlia - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Henzl, Sandra - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Kim, Jihoon - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Kim, Jong Kyoung - *Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, Daegu, Korea*
Kim, Somi - *Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, Daegu, Korea*
Koo, Bon-Kyoung - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Lee, Heetak - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Lee, Ji-Hyun - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Pauli, Andrea - *Pauli Lab, Institute of Molecular Pathology, Vienna, Austria*
Szép-Bakonyi, Réka - *Koo Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna, Austria*
Theussl, Hans Christian - *Transgenic, Institute of Molecular Pathology, Vienna, Austria*

Abstract: Image-based lineage tracing has allowed the interrogation of adult tissue turnover kinetics and lineage potential of different cell populations. Based on the multicolor reporter systems, several mosaic genetic systems have been developed. Previously, we reported Red2Onco, which ectopically express mutated oncogenes together with the RFP, thereby allowing the dissection of expansion kinetics and neighboring effects of oncogenic clones. In the current study, we report Red2Flpe, a new mosaic knockout system with multicolor reporters for both mutant and wildtype cells. Red2Flpe shows efficient and specific recombination in the RFP+ clones both in vitro and in vivo. To facilitate new conditional knockout (cKO) mouse line generation, we have developed a Short Conditional intrON (SCON) technology that is suitable for one-step cKO allele generation via zygote injection. SCON is compatible with both Cre/loxP- and Flp/frt-based cKO recombination systems. SCON shows no signs of hypomorphism prior recombination, while knockout is efficiently induced upon recombination. Utilizing Red2Flpe and Sox2-SCONfrt, we investigated the functions of Sox2 in the adult esophagus in which Sox2 has been thought to be crucial for stem cell maintenance

and tissue turnover. However, mosaic Sox2 knockout clones in a wildtype environment persist after >2 months of lineage tracing, suggesting a non-essential role of Sox2 in maintaining stem cell characteristics. Clone size of Sox2-KO cells are smaller compared to the wildtype clones in the same tissues, which indicates a lowered fitness upon Sox2 knockout. We performed single-cell RNA sequencing (scRNAseq) of sorted cells and found differences in proliferation characteristics of wildtype and Sox2-KO cells. We conclude that Sox2 is not an essential stemness marker, but a regulator of proliferation kinetics in basal cells. Overall, we have constructed a toolkit for in vivo mosaic knockout studies that is suitable for clonal tracing with internal controls and a pipeline for one step generation of cKO alleles.

Keywords: Multicolor reporter, Mosaic conditional knockout, Adult stem cell

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QUANTITATIVE METHODS FOR EVALUATION OF CELL HEALTH DURING DIFFERENT STAGES OF CELL PROCESSING

Kandell, Jennifer - *Pharma Services Group, Thermo Fisher Scientific, La Jolla, CA, USA*
Snyder, Richard - *Pharma Services Group, Thermo Fisher Scientific, Alachua, FL, USA*
Lakshmipathy, Uma - *Pharma Services Group, Thermo Fisher Scientific, San Diego, CA, USA*

Abstract: Cell counting is one of the most important measurements used to determine cell health in biomanufacturing. Quantitative measurements of cell health are especially important when working with different cell types, where variation between cell types and their intended uses can be further amplified by user subjectivity. Automated cell counting system offer the ability to standardize cell counting processes and increase efficiency, when compared to traditionally used manual counting methods. However, these systems can be affected by the physical properties of different cell types and interfering agents in the cell solution, including cell debris and magnetic beads. These factors make it challenging to develop a consistent method that offers a high level of confidence in the results. Several initiatives from standards development organizations have attempted to address this critical need for standardized cell counting. In this study, flow-based and image-based methods were used to quantitatively measure cell counts and viability. Both adherent and suspension cells were used, from primary tissue sources and established cell lines. The cells used were of different sizes, circularities, and diameters. Each cell type was evaluated at different stages of cell processing. Results from this study define a systematic approach that enables the identification of counting method and parameters that are best suited for specific cell types and workflows that ensures accuracy and consistency.

Keywords: Cell counting, Cell health, Biomanufacturing

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NOVEL T-CELL ACTIVATION REAGENT TO FACILITATE T-CELL THERAPY

Patel, Daksha - *CPBU, Genscript, Piscataway, NJ, USA*
Bogoev, Roumen - *CPBU, Genscript, Piscataway, NJ, USA*
Qian, Hong - *CPBU, Genscript, Nanjing, China*
He, Ruina - *CPBU, Genscript, Nanjing, China*

Abstract: A rapidly emerging immunotherapy approach is called adoptive cell transfer (ACT): collecting and using patients' own immune cells to treat their cancer. There are several types of ACT but, thus far, the one that has advanced the furthest in clinical development is called CAR T-cell therapy. CAR T cells are the equivalent of "giving patients a living drug," We developed innovative, uniform biocompatible, biodegradable anti-CD3 -CD28 coupled EnceedTM T Cell Activation reagent, which allow feeder cells free (antigen-presenting cells) or antigen free, activation and expansion of T cells from PBMCs and of enriched T cell populations, while maintaining high viability. Process is simple and robust, no need to remove nano beads during expansion and activation. Genscript EnceedTM T Cell Activation beads showed a comparable or even higher activation signal compared to activation beads from leaders on the market and these methods can be scaled up to support clinical-level T-cell manufacturing and assay development

Keywords: T Cell Activation, feeder cells free, T-CELL THERAPY, nano beads

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LABEL-FREE MACHINE VISION-BASED CELL SORTING FOR TISSUE ENGINEERING

Ugarte, Fernando - *Drug Discovery, Thinkcyte, San Carlos, CA, USA*
Asawa, Yukiyo - *Division of Tissue Engineering, The University of Tokyo Hospital, Tokyo, Japan*
Hikita, Atsuhiko - *Department of Tissue Engineering, The University of Tokyo Hospital, Tokyo, Japan*
Hoshi, Kazuto - *Department of Oral-maxillofacial Surgery, The University of Tokyo Hospital, Tokyo, Japan*
Kawamura, Yoko - *Microfluidics, Thinkcyte, Tokyo, Japan*
Nomaru, Hiroko - *Cell therapy, Thinkcyte, Tokyo, Japan*
Shimizu, Reina - *Department of Sensory and Motor System Medicine, The University of Tokyo, Japan*
Teranishi, Kazuki - *Drug Discovery, Thinkcyte, San Carlos, CA, USA*

Abstract: In regenerative medicine, purification of the target therapeutic cells is often required, and sorting by flow cytometry requires cell labeling with high quality antibodies or the insertion of a reporter gene to identify the target cells, both of which are tedious and expensive procedures. We have developed a high-throughput machine vision-based cell sorting technology based on label-free ghost cytometry (LF-GC), which enables classification and sorting of cells using machine-predicted labels based on compressive imaging information without image reconstruction. In this system, the waveform that contains image information is recorded when the cell passes through a structured illumination, and then the classifier is generated by biologically supervised machine learning. Using the labels predicted by a machine-learning-based model, the cells are classified in a short time and sorted at high throughput. Cartilage regeneration is one of the targets for regenerative therapy because of poor regenerative potential. A previous study showed that the human chondrocytes labeled with CFSE exhibited a wide range of fluorescent intensities, and demonstrated that chondrocytes with the rapid proliferation (CFSE low) showed more matrix production potential than the other cells (CFSE high). To evaluate if LF-GC is applicable to the enrichment of high matrix producing chondrocytes, we analyzed CFSE-stained human chondrocytes on LF-GC. We defined rapid proliferating cells as the low 30% of CFSE intensity, and slow proliferating cells as the other 70%. Our data generated

a LF-GC classifier based on support vector machine (SVM) with the area under the receiver operating characteristic curve (AUC) of 0.86. Using this LF-GC classifier, we enriched the rapid proliferation cell population from 33.6% to 76.8%. After three weeks of culturing the sorted cells as pellets, the highly proliferative cells (CFSE low) significantly accumulated more glycosaminoglycans (GAGs) compared to the control samples, demonstrating their higher extracellular matrix production potential. Here, we demonstrated LF-GC's potential to purify the desired cells without any staining, suggesting that this could be a new effective tool for label-free and selective cell isolation and purification in regenerative medicine.

Keywords: Ghost cytometry, label-free sorting, machine learning

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DEVELOPING THE NEXT GENERATION OF IPSC-BASED HIGH-THROUGHPUT CRISPR SCREENING PLATFORMS FOR INSIGHTS INTO HUMAN DISEASE BIOLOGY

Przybyla, Laralynne - *Biochemistry and Biophysics, University of California, San Francisco, CA, USA*
Esanov, Rustam - *Laboratory for Genomics Research, GlaxoSmithKline, San Francisco, CA, USA*
Shevade, Kaivalya - *Laboratory for Genomics Research, University of California San Francisco, CA, USA*
Sevim, Volkan - *Laboratory for Genomics Research, GlaxoSmithKline, San Francisco, CA, USA*
Li, Marcus - *Laboratory for Genomics Research, University of California San Francisco, CA, USA*
Federman, Scot - *Laboratory for Genomics Research, University of California ,San Francisco, CA, USA*
Peddada, Sailaja - *Laboratory for Genomics Research, University of California San Francisco, CA, USA*
You, Kwontae - *Laboratory for Genomics Research, GlaxoSmithKline, San Francisco, CA, USA*
Yang, Angela - *Laboratory for Genomics Research, GlaxoSmithKline, San Francisco, CA, USA*
Shafer, Shawn - *Laboratory for Genomics Research, GlaxoSmithKline, San Francisco, CA, USA*

Abstract: In the decade since genome-wide CRISPR screens were first described they have become a routine tool for identifying genes with key roles in biological processes and disease phenotypes. In parallel, advances in cellular biology have allowed researchers to move away from abnormal cancer and immortalized cell lines towards iPSC-derived models that are more representative of human biology. However, we currently lack systematic strategies for generating iPSC-derived cells at the scale and efficiency required for use in genome-wide CRISPR screens, limiting the broader applicability of these models. To address this shortcoming, our lab has developed a robust and universal assay for validating functional CRISPR iPSC models at single cell resolution and has demonstrated long-term maintenance of CRISPRi and CRISPRa machinery with robust and specific induction in iPSCs and differentiated cells. Using these foundational tools, we developed systematic profiling approaches to identify candidate transcription factors for lineage-specific deterministic iPSC differentiation compatible with high-throughput functional genomic screening. In parallel, we developed strategies to systematically identify factors whose levels can be modulated to enhance transduction efficiency of iPSC-derived cells. To ensure that our iPSC-based model systems both accurately represent the phenotype of interest and exhibit endogenous expression levels of disease-relevant genes, we developed co-culture screens to model cellular interactions and organoid-based screens to replicate human tissue biology. Together, these approaches allow us to perform screens across protein-coding and non-coding regions in iPSC-derived cells across diverse areas of disease biology. These platforms were developed at the Laboratory for Genomics Research (LGR), a collaborative functional genomics center focused on building and applying CRISPR/Cas-based technologies to advance discovery of genetic and mechanistic links to human disease. By developing tools to overcome the challenges of using iPSC-derived cells in CRISPR screening platforms, our goal is to make large-scale pooled screens on human iPSC-derived cells routine practice to enable target discovery in sophisticated model systems that faithfully recapitulate human disease biology.

Funding Source: GlaxoSmithKline
Keywords: CRISPR screening approaches, human disease models, iPSC differentiation platforms

TOPIC: PLURIPOTENT STEM CELLS

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THE HEPATITIS B VIRUS X PROTEIN (HBX) IMPROVES IPSC DERIVATION FROM 'DIFFICULT TO REPROGRAM' SAMPLES AND CAN SUBSTITUTE FOR MYC IN YAMANAKA'S COCKTAIL

Sanal, Madhusudana Girija M. - *Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, New Delhi, India*
Gupta, Sarita - *Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, New Delhi, India*
Saha, Rahul - *Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, New Delhi, India*
Vats, Nisha - *Molecular and Cellular Medicine, Institute of Liver and Biliary Sciences, New Delhi, India*
Sarin, Shiv - *Hepatology, Institute of Liver and Biliary Sciences, New Delhi, India*

Abstract: HBx is a multifunctional and promiscuous protein coded by the Hepatitis B virus which is involved in various cellular processes such as proliferation, cell survival/apoptosis, and histone methylation. HBx is known to induce markers of 'stemness' in liver cancer cells. It is possible that HBx may help the virus to 'reprogram' the epigenetic landscape of hepatocytes to facilitate its replication and in this process, cells mutate to form hepatocellular carcinoma. We hypothesized that we could take advantage of the stemness-inducing properties of HBx if it can facilitate the generation of pluripotent stem cells from somatic cells. Oct4, Sox2, Klf4, and c-Myc are the transcription factors (Yamanaka factors) used to generate induced pluripotent stem cells (iPSC). We electroporated control fibroblasts and high passage (difficult to reprogram samples) with episomal plasmids expressing a) Yamanaka's factors with and with-out HBx b) Yamanaka's factors minus Myc plus HBx (substituted Myc with the plasmid expressing HBx) and maintained the cells as per the standard iPSC derivation protocol. We found that the addition HBx not only improves the derivation of iPSC using Yamanaka's factors but also it can substitute the transcription factor Myc. HBx also improves the efficiency of generation of iPSC from 'difficult samples' (high passage fibroblasts) and the increase in efficiency was statistically significant ($p < 0.05$). Not only that our observations have some practical value in improving the efficiency of pluripotent stem cell derivation from somatic cells but also give some insights into the possible mechanisms of

liver carcinogenesis and the actions of HBx. We have for the first time demonstrated in-vitro that Hbx can upregulate Oct3/4 and enhance the generation of iPSC. We have also shown that the iPSC generated using Hbx can be differentiated to all three germ layers. We have also differentiated them into hepatocyte-like cells. HBx DNA sequence or HBx protein was not detectable in iPSC generated using HBx. Further, upon differentiation pluripotency markers were undetectable.

Funding Source: 1) Science and Engineering Research Board (grant #ECR/2015/000275) for limited financial support
2) Department of Biotechnology, Ministry of Science and Technology, India (grant #BT/PR15116/MED/31/334/2016) Government of India
Keywords: HBx / Hepatitis B Virus X protein, iPSC/induced Pluripotent Stem Cells, reprogramming efficiency

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PRIME INDUCED NUCLEOTIDE ENGINEERING USING A TRANSIENT REPORTER FOR EDITING ENRICHMENT (PINE-TREE)

Kostes, Warner - *School for Engineering of Matter, Transport and Energy, Arizona State University, Scottsdale, AZ, USA*
Frisch, Carlye - *School for Engineering of Matter, Transport & Energy, Arizona State University, Tempe, AZ, USA*
Galyon, Brooke - *School for Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*
Whitman, Brycelyn - *School for Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, USA*

Abstract: Prime editing is a versatile gene editing method that mediates targeted insertions and deletions and can perform all 12 types of point mutations. Furthermore, prime editing is compatible with many therapeutically relevant cell types and organisms that are traditionally less amenable to editing when using CRISPR/Cas9 or base editors. With over 75,000 known human pathogenic genetic variants, prime editing is an attractive option to correct these mutations that remain difficult to edit with current genome editing methods. Prime editing technology features the use of a Cas9 nickase fused to a reverse transcriptase. With this complex is a prime editing guide RNA (pegRNA) that both specifies the genomic target site and contains the genetic sequence for the desired edit. We aim to enhance prime editing with our transient reporter for editing enrichment (TREE) technology to develop a method for the rapid generation of clonal isogenic cell lines for disease modeling. TREE uses an engineered BFP variant that upon a C-to-T conversion will convert to GFP after target modification. When paired with fluorescence-activated cell sorting (FACS), this BFP-to-GFP conversion assay allows for the isolation of base edited cell populations via a fluorescent reporter of editing. Prime induced nucleotide engineering using a transient reporter for editing enrichment (PINE-TREE) features the pairing of prime editing with TREE technology. In this investigation, we employed flow cytometry to optimize the episomal editing efficiency of PINE-TREE in hiPSCs. Furthermore, we showed increased single nucleotide editing efficiencies across 5 genomic sites in hiPSCs when using PINE-TREE compared to a reporter of transfection (RoT). Additionally, we compared the editing efficiency of PINE-TREE to a RoT when performing small insertions and deletions in hiPSCs. We used FACS to sort single cells and compare editing efficiencies of PINE-TREE to RoT and performed an off-target analysis on clones. Lastly, we inserted a FLAG-tag sequence downstream of a gene as a proof of concept for precise insertion of a larger nucleotide sequence in hiPSCs. This work will serve as a valuable resource to enhance prime editing and accelerate the generation of clonal isogenic cell lines for disease modeling.

Keywords: prime editing, gene editing, human pluripotent stem cells

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ESTABLISHING TRANSDUCTION METHODOLOGIES IN BETA CELLS DERIVED FROM HPSCS

Marquez, Erica L. - *Department of Medicine, Washington University, Saint Louis, MO, USA*
Velazco-Cruz, Leonardo - *Department of Medicine, Washington University in St. Louis, MO, USA*
Augsornworawat, Punn - *Department of Medicine, Washington University in St. Louis, MO, USA*
Miller, Julia - *Department of Medicine, Washington University in St. Louis, MO, USA*
Millman, Jeffrey - *Department of Medicine, Washington University in St. Louis, MO, USA*

Abstract: Genetic modulation of novel genes is an essential tool for elucidating mechanism of human pluripotent stem cell (hPSC) differentiation and is a strategic approach in identifying the role of key factors essential to the differentiation process. Our lab has developed approaches allowing for consistent, high-titer virus that is curated for SC-β cells using a single, modifiable lentiviral system. We have applied this platform to study MAFA, a transcription factor that is essential to maturation and function in late-stage differentiation if hPSC-derived β (SC-β) cells. The MAFA gene is responsible for regulation of glucose-stimulated insulin secretion that is essential for homeostasis. Here we describe the system resulting in multi-fold Knock Down (KD) and overexpression. Our lentivirus methods have proven successful introducing these genes edits on a variety of cell lines including SC-β cells. This aims to identify methods for the insertion and deletion of target sequences utilizing lentivirus as a vector for shRNA, CRISPR, and ORF sequences in SC-β cells in a reliable and efficient system. The specialized development of this process has allowed for a consistent, high-titer lentivirus that reliably KD and overexpress target genes as measured through qPCR. The methods used allow for the application of gene edits to study SC-β cell biology and other differentiation systems of interest to the biomedical sciences.

Funding Source: This work was funded by NIH (5R01DK114233), JDRF Career Development Award (5-CDA-2017-391-AN), and Department of Biomedical Engineering and School of Medicine at Washington University in St. Louis
Keywords: Lentivirus, Genetic Modification, Methodology

536

DEVELOPING A NOVEL WNT MIMETIC USING TCDB

Anjakos, David - *Department of Biology, San Diego State University, Chula Vista, CA, USA*
Kenner, Nate - *Department of Biology, California State University San Marcos, CA, USA*
Willert, Karl - *Department of Cellular and Molecular Medicine, University of California San Diego, CA, USA*

Abstract: The creation of induced pluripotent stem cells (iPSC) has opened new opportunities and possibilities in regenerative medicine and personalized medicine, such as tissue engineering, disease modeling, and drug testing. To facilitate these new techniques, scientists have to unravel and dissect the many signals that induce specific genes expression programs controlling distinct cellular fates. Wnt (pronounced wint) genes encode a family of proteins critical to stem cell function, cell fate, cell proliferation, and cell migration. Mutations in Wnt genes and associated downstream signaling components have been linked to a host of diseases, such as cancer, osteoporosis, and developmental abnormalities like Robinow syndrome, to name a few. Wnt proteins are lipid-modified secreted growth factors with a limited range of activity. Unfortunately, due to Wnts' lipid modification, their isolation and purification require detergents, which complicate research and therapeutic applications. To solve this issue, our lab is utilizing antibody engineering to generate Wnt mimetics that are capable of activating downstream signaling. Previously our lab developed a Wnt mimetic that activates a single Wnt signaling pathway by binding with Frizzled 7 (FZD7) and the coreceptor, LRP6. We are expanding on this technology by generating Wnt mimetics that target multiple FZD receptors. Our next mimetic utilizes the toxin B (TcdB) protein of Clostridium Difficile, which preferentially binds FZD 1, 2, and 7. We have confirmed the production of our protein in HEK-293 cells via Western Blot, and are now attempting to produce a large amount of our protein in CHO cells for further analysis. If we are successful, this protein will be a great tool for Wnt's many research fields and will enable applications in the regenerative medicine space.

Keywords: Wnt, iPSC, TcdB

TOPIC: NT - GENERAL

538

URINARY-DERIVED 3D UROTHELIAL CARCINOMA ORGANOIDS: A PROMISING TOOL FOR THE DIAGNOSIS AND INDIVIDUALIZED THERAPY OF BLADDER CANCER

Wickham, Hannah M. - *Iowa State University, Ankeny, IA, USA*
Sahoo, Dipak - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Zdyrski, Christopher - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Gabriel, Vojtech - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Wickham, Hannah - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*
Bourgois-Mochel, Agnes - *Veterinary Clinical Sciences, Iowa State University, Ames, IA, USA*
Lucien, Fabrice - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Frank, Igor - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Cheville, John - *Department of Urology, Mayo Clinic, Rochester, MN, USA*
Allenspach, Karin - *SMART Translational Medicine, Iowa State University, Ames, IA, USA*

Abstract: Muscle-invasive bladder cancer (MIBC) is associated with a poor survival rate of 40% at 5 years follow-up. Current standard-of-care in the management of bladder cancer relies on platinum-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy. However, even with this aggressive course of treatment, up to 50% of patients with MIBC fail to respond to chemotherapy. A key variable that contributes to poor patient outcomes lies in our current inability to predict therapeutic response to NAC in light of the phenotypic and molecular heterogeneity of MIBC tumors. There is, therefore, a significant unmet clinical need to develop novel biological tools that can refine patient stratification and accurately predict response to chemotherapy in MIBC. A promising technology in this regard exists in the culture of 3D patient-derived tumor organoids (PDOs) that can mimic cell-to-cell and cell-to-matrix interactions, in vivo metabolic environment, and diffusion kinetics of therapeutic drugs. However, current methods for expansion of PDOs rely on resected tumor samples; a procedure which is inherently invasive and further hampers clinical applications at the bedside. Instead, we propose, for the first time, that the culture of PDOs can be established from urine samples obtained non-invasively in MIBC patients. Leveraging our expertise in the culture and maintenance of canine enteroids/colonoids, 3D organoids were successfully derived from 10 mL of urine in 2/2 patients, using a modified version of our standard protocol, including additional growth factors FGF2, FGF7, and FGF10. Immunohistochemistry staining for Gata 3, p63 and Pax 8 (Gata 3+, p63+, Pax 8-) confirmed the urothelial (vs. renal i.e., Pax8+) origin of 3D organoids. The malignant nature of urinary PDOs was assessed by cytomorphological evaluation, along with immunostaining for uroplakin-2 (urothelium-specific protein over-expressed in bladder cancer). Collectively, these preliminary findings suggest that 3D PDOs can be derived non-invasively from urine and recapitulate expression of key markers of MIBC tumors. This platform provides an opportunity for bedside testing of NAC to improve treatment response and clinical outcome in patients with bladder cancer.

Funding Source: Barry Cancer Research Foundation
Keywords: Bladder Cancer, 3D Organoids, Precision Medicine

TOPIC: EPITHELIAL_SKIN

854

A NOVEL CO-CULTURE SYSTEM OF MULTIPLE CELL TYPES FOR MIMICKING SKIN MICROENVIRONMENT

Jeong, Yun-Mi - *Department of Mechanical Engineering, Korea Polytechnic University, Siheung-si, Korea*
Jin, Songwan - *Department of Mechanical Engineering, Korea Polytechnic University, Siheung-si, Korea*
Kang, Chanhan - *Department of Mechanical Engineering, Korea Polytechnic University, Siheung-si, Korea*
Yun, Won-Soo - *Department of Mechanical Engineering, Korea Polytechnic University, Siheung-si, Korea*

Abstract: Recent advances have been made toward developing three-dimensional (3D) in vitro human skin models, including skin characteristics in terms of epidermal and dermal structures, barrier functions, cell migration, and cell-to-cell interaction in the 3D microenvironment. However, current 3D in vitro human skin models have been a limitation that monitors long-term real-time for validating in vitro, at least, with the co-culture of two main types of skin cells. To overcome these limitations, we investigated whether decellularized bioscaffolds of non-human origin provide a naturally derived microenvironment for skin-associated cells to be adhesion, migration, growth, and differentiation their great merits. The decellularized bioscaffolds of non-human origins were made

by pig tissue with muscle/fat based on combination methods of chemical and physical tools. Hematoxylin and eosin (H&E), scanning electron microscopy, Hoechst 3342 staining, and genomic material contents demonstrate the complete decellularization of bioscaffolds. A novel co-culture system of skin microenvironment was fabricated an epidermal layer and dermal layer seeded with a clip- mold and multiple cell-based collagen solution. To prove our hypothesis, CDy6 mitotic tracker probe-stained fluorescence imaging, and qRT-PCR analysis show that 3D in vitro decellularized bioscaffold-mediated skin models are suitable conditions for the maintenance and survival rate of compatible different cell lines. In conclusion, our findings can be useful for a new intelligent and more efficient safety assessment, based on in vitro testing into a predictive safety assessment of biomaterials.

Funding Source: 2020R1IA1A0105458; 2017R1A6AIA03015562
Keywords: CO-CULTURE SYSTEM, MULTIPLE CELL TYPES, SKIN MICROENVIRONMENT

TOPIC: KIDNEY

856

P-SELECTIN TARGETED EXTRACELLULAR VESICLES PROTECTS AGAINST ACUTE ISCHEMIC KIDNEY INJURY BY ENDOTHELIAL CELL SPECIFICALLY BINDING

Zhang, Kaiyue - *School of Medicine, Nankai University, Tianjin, China*
Chen, Shang - *School of Medicine, Nankai University, Tianjin, China*
Huang, Haoyan - *School of Medicine, Nankai University, Tianjin, China*
Liu, Yue - *School of Medicine, Nankai University, Tianjin, China*
Li, Zongjin - *School of Medicine, Nankai University, Tianjin, China*

Abstract: Prevention of acute kidney injury (AKI) to chronic kidney disease (CKD) progression, which has a gradient-response relationship with the severity of AKI, is hampered by challenges in its early diagnosis and targeted therapy. Here, we identified P-selectin as a biomarker of AKI severity and developed P-selectin binding peptide (PBP) engineered extracellular vesicles (PBP-EVs) with imaging and therapeutic functions for indicating AKI severity at an early stage and preventing AKI to CKD progression. Our results showed that PBP-EVs exhibited the selective targeting tendency to injured kidneys, while providing the spatiotemporal information for early diagnosis of AKI by quantifying the P-selectin in kidneys. Meanwhile, PBP-EVs revealed superior nephroprotective functions in accelerating renal repair and inhibiting fibrosis via reducing inflammation infiltration, improving reparative angiogenesis, and ameliorating maladaptive repair of the renal parenchyma. In conclusion, PBP-EVs as an AKI theranostic system we designed in this study provided a spatiotemporal diagnosis in the early stages of AKI to help guide personalized therapy and exhibited superior nephroprotective effects, offering proof-of-concept data to design EV-based theranostic strategy for promoting renal recovery and further preventing the AKI to CKD progression.

Keywords: extracellular vesicles, acute kidney injury, P-selectin binding peptide (PBP)

TOPIC: NEURAL

858

A NON-VIRAL INDUCIBLE TRANSGENIC SYSTEM USED FOR TRANSCRIPTION FACTOR BASED DIRECTED DIFFERENTIATION OF NEURONS AND GLIA

Linesch, Paul - *Biomedical Sciences, Cedars-Sinai Graduate School, West Hollywood, CA, USA*
Kobritz, Naomi - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Yang, Amy - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Diaz-Guijarro De Las Heras, Henry - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Park, Hannah - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Rincón Fernández Pacheco, David - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Mattis, Virginia - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Breunig, Joshua - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: A directed differentiation technique for the Central Nervous System (CNS) would be a novel method to study mature populations of specific CNS cell types in vitro and could serve as a transplant therapy for replenishing lost post-mitotic cells in neurodegenerative diseases. In this experiment, we transiently express a mixture of key developmental transcription factors using a novel nonviral inducible transgenic system and preliminary results indicate we can directly differentiate neurons and other CNS cell types. Using an inducible cocktail of transcription factors and microRNA (CTIP2, DLX2, miR-9 & 124) we can direct the differentiation of stem cells into Medium Spiny Neurons (MSN). A transgenesis method developed by the Breunig Lab named 'MADR', Mosaic Analysis with Dual recombinase-mediated cassette exchange, uses two recombinase (Cre & FlpO) to integrate a donor DNA cassette between LoxP and FRT sites. This inducible donor cassette has two parts, a constitutively active side expressed under a CAG promoter, and a doxycycline (DOX) inducible "tet-on" element to express a cassette containing genes of interest. We have successfully validated nucleofection using primary mouse neural stem cells (NSCs) from mTmG mice both in vivo and in vitro, as well as in human iPSCs. In vitro primary mouse NSCs displayed a morphological change resembling a neuronal cell type and genomic analysis indicates an increase in pro-neuronal and synaptic genes. Current experiments involve a stably transfected iPSC line to further test differentiation in vitro and transplanting these cells into NOD-SCID mice to investigate the integration of differentiated cells. Additional in vivo experiments include using postnatal electroporation to transfect radial glia progenitor cells lining the ventricle in mice at postnatal day 1-2 and inducing expression after 30 days with doxycycline containing food. After two weeks of DOX administration, neuronal looking projections begin to be seen in SMFP-MYC-bright reporter positive cells. This data indicates MADR can be used to tightly control the directed differentiation of stem cells, allowing us to model diseases in the dish by differentiation mature cell types and explore transplantation studies to treat various neurodegenerative diseases.

Funding Source: NIH R03NS101529 Cedars-Sinai NIH 1T32GM118288 Cedars-Sinai Graduate School

Keywords: Directed Differentiation, Neuronal Stem Cell Differentiation, Neuronal Development

TOPIC: NT - GENERAL

860

POTENT MESENCHYMAL STROMAL CELLS WITH DESIRED ATTRIBUTES CAN BE IDENTIFIED USING STATISTICAL EVALUATION OF PUTATIVE CRITICAL QUALITY ATTRIBUTES

Robb, Kevin P. - *Institute of Biomedical Engineering, University of Toronto, ON, Canada*
Audet, Julie - *Institute of Biomedical Engineering, University of Toronto, ON, Canada*
Gandhi, Rajiv - *Osteoarthritis Research Program, Division of Orthopedic Surgery, Schroeder Arthritis Institute, University Health Network, Toronto, ON, Canada*
Viswanathan, Sowmya - *Osteoarthritis Research Program, Division of Orthopedic Surgery, Schroeder Arthritis Institute, University Health Network, Toronto, ON, Canada*

Abstract: Adipose-derived mesenchymal stromal cells [MSC(AT)] display immunomodulatory and angiogenic properties but an improved understanding of quantitative and functional critical quality attributes (CQAs) is needed for their effective clinical translation. We applied statistical methods to evaluate a matrix of putative CQAs for MSC(AT) immunomodulatory and angiogenic properties, with consideration for culture methods (3D aggregate, 2D hypoxic and 2D normoxic), donor heterogeneity (N=5 human donors), and licensing conditions (licensed with pro-inflammatory cytokines, or unlicensed). We measured curated genes (59), secreted factors (10), in vitro monocyte/macrophage (MΦ) polarization and in vitro angiogenesis to evaluate putative CQAs. Unbiased hierarchical clustering of MSC(AT) immunomodulatory and angiogenic markers (genes and secreted factors) showed these were modulated by culture methods, donor and licensing. 3D MSC(AT) had significantly higher expression of immunomodulatory markers and lower expression of angiogenic markers relative to 2D culture methods. Corroborating these data, 3D MSC(AT) significantly promoted a more inflammation-resolving MΦ phenotype in vitro as demonstrated by principal component analysis of MΦ gene and protein expression panels. Hypoxic MSC(AT) showed intermediate immunomodulatory properties. Regression analyses of the putative CQAs revealed a significant inverse relationship between immunomodulatory functional properties and expression of 7 angiogenic genes (R2 range: 0.27-0.55), suggesting an inverse interplay between these MSC(AT) attributes. Desirability profiling of the putative CQAs allowed us to rank the MSC(AT) by preferred properties; for example, 3D MSC(AT) ranked highest for immunomodulatory function. Interestingly, licensed conditions for MSC(AT) showed different desirability rankings compared to unlicensed conditions, where donor heterogeneity predominated. In ongoing studies, in vitro angiogenic assays are being performed to further explore MSC(AT) putative CQAs. Taken together, the statistical approach enabled combinatorial analysis of the matrix of in vitro assays to provide putative CQAs that can be used to prospectively screen potent MSC donors and culture conditions to optimize for desired MSC functionality.

Funding Source: This project received funding from the CIHR (PJT-166089) and OIRM New Ideas grant awarded to SV. Salary support for KPR is provided by a NSERC CGSD scholarship.

Keywords: mesenchymal stromal cell, critical quality attribute, statistical modelling

TOPIC: PLURIPOTENT STEM CELLS

862

SUBCULTURE METHOD OF HUMAN IPS CELLS IN SUSPENSION CULTURE USING A CELL DISPERSION DEVICE

Wada, Masanori - *Research and Development, ABLE Corporation, Tokyo, Japan*
Matsuura, Katsuhisa - *Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Shinjuku-ku, Japan*

Abstract: The stirred suspension culture using a bioreactor system is an efficient method for the large-scale expansion of human iPS cells. In suspension culture, human iPS cells form aggregates that must be dispersed into single cells to scale up. In the small-scale cell dispersion step, enzyme solution treatment and pipetting are performed manually. This process was prone to individual procedure differences and prevented expansion into large-scale cultures. We have developed a reactor equipped with a cylinder that rotates at high speed to refine manual cell dispersion (cell dispersion device). The Taylor vortex flow generated by the rotation of the cylinder was effective for cell dispersion. There are two types of cell dispersion devices, 20 mL (small scale) and 100 mL (bench top scale), each of which could process 100 million cells and 1 billion cells. Aggregates of human iPS cells prepared using our suspension culture reactor were dispersed into single cells using a cell dispersion device. Dispersion of iPS cell aggregates using this device required no enzymes, only chelating agents. The cell suspension thus prepared was seeded in a larger scale reactor to form aggregates and proliferate similarly. As a result, scale-up suspension culture from 20 million cells to 10 billion cells could be performed in 12 days using 100 mL, 500 mL, and 5000 mL suspension culture reactors.

Funding Source: Project Focused on Developing Key Evaluation Technology: Acceleration of Regenerative Medicine Seeds Development, AMED, Japan
Keywords: Human iPS cells, Suspension culture, Scale-up culture

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

602

HUMAN DENTAL PULP-DERIVED MSCS HAVE POTENTIAL TO RESCUE OXIDATIVELY DAMAGED MSCS THROUGH TRANSFER OF HEALTHY MITOCHONDRIA

Oh, Seong-Ju - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Choe, Yong-Ho - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Kim, Saet-Byul - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Han, Jang-Ho - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Jo, Chan-Hee - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Lee, Sung-lim - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*
Rho, Gyu-jin - *Veterinary Obstetrics, College of Veterinary Medicine, Gyeongsang National University, Jinju-si, Korea*

Abstract: Mesenchymal stem cells (MSCs) have been widely used for clinical therapies for tissue regeneration. Current studies demonstrated that MSCs can recognize oxidative stress in somatic cells and rescue the metabolic state of the injured cells by mitochondrial transfer. However, there is limited understanding of the mitochondrial transfer from dental pulp-derived MSCs (DPSCs). In this study, we investigated whether healthy DPSCs could transfer their mitochondria into oxidatively damaged DPSCs for restoring mitochondrial functions. To establish an in vitro oxidative stress model, DPSCs were treated with different concentrations (100, 200, 300, 400, and 500 μM) of hydrogen peroxide (H2O2) for 3 h. We have comparatively analyzed the cell survival rate, proliferation ability, and reactive oxygen species (ROS) level, and 300 μM of H2O2 was verified as an appropriate concentration to induce oxidative stress without cell death in DPSCs. To detect mitochondrial transfer of DPSCs, H2O2-treated DPSCs were labeled with CFSE, and mitochondria of healthy DPSCs was labeled with MitoTracker-DeepRed (MTDR). Both of DPSCs were then co-cultured at a 1:1 ratio for 24 h. After co-culture, the change in ROS level by mitochondrial transfer was measured through MitoSOX analysis. Fluorescence microscopy and flow cytometry results showed successful mitochondrial transfer from healthy DPSCs into H2O2-treated DPSCs (CFSE+ MTDR+ cells). In MitoSOX analysis, ROS level of H2O2-treated DPSCs with mitochondria of healthy DPSCs (CFSE+ MTDR+ cells) were significantly ($P < 0.05$) reduced as compared with non-co-cultured H2O2-treated DPSCs. In conclusion, this study showed mitochondrial transfer of healthy DPSCs can reduce oxidative stress in H2O2-treated DPSCs. It suggests that the mitochondrial transfer of healthy DPSCs can rescue the damaged MSCs of patients, in oxidative stress-related diseases including Alzheimer's disease, diabetes, and cancer.

Funding Source: This study was supported by a grant from the National Research Foundation (NRF) of Korea, funded by the government of the Republic of Korea (grant #. NRF-2021R1A2C1007054) and from Stem Centric Co. Ltd. in the Republic of Korea.
Keywords: Human dental pulp-derived mesenchymal stem cells, mitochondrial transfer, oxidative stress

TOPIC: EARLY EMBRYO

604

EXPLORING THE POTENTIAL OF NEUROMESODERMAL PROGENITORS TO MAKE NEURAL PROGENITORS OF SPECIFIC AXIAL IDENTITY FOR SPINAL CORD GRAFTING

Alonso Torrens, Aaron - *Centre for Regenerative Medicine, The University of Edinburgh, UK*
Wilson, Val - *Centre for Regenerative Medicine, The University of Edinburgh, UK*

Abstract: After an injury, axons in the mammalian spinal cord fail to regenerate, and the spinal cord cannot repair itself. A promising approach to improving recovery after spinal cord injury (SCI) is the transplantation of neural stem or progenitor cells (NPCs) derived from pluripotent cells into the injury site. However, there are significant obstacles to this potential therapeutic approach. Grafted NPCs must survive and integrate appropriately into the host; however, in practice, successful transplantation is dependent on several factors. One known cause of failure of successful integration is a mismatch between the anteroposterior identity of the grafted cells and the host site. In addition, it has until recently not been possible to generate in vitro posterior NPCs corresponding to the thoracic/ lumbosacral spinal cord. The production of neuromesodermal progenitors (NMP) cells in vitro has now opened promising avenues towards generating functional posterior NPCs. To determine functional integration, survival, neurite outgrowth and pathfinding of cells after grafting, we differentiated Epiblast stem cells (EpiSCs) towards NPCs of anterior (NA), hindbrain (NH) and posterior thoracic spinal cord identity (NS) and grafted them into E10.5 mouse embryos at homotopic and heterotopic locations. We show that GDF11 can drive cells towards a sacral identity, and show that the timing of GDF11 treatment during NMP differentiation is critical for optimal expression of sacral/caudal Hox genes. We show that grafted NPCs survive and integrate into the host. Furthermore, NPCs retain their neural and anteroposterior axial identity when grafted into the neural tube. This suggests that cells' axial identity is already fixed in the grafted NPCs, highlighting the importance of generating cells of defined anteroposterior axial identities as potential cellular therapies.

Keywords: Neuromesodermal progenitors (NMPs), Neural progenitor cells (NPCs) of specific axial identity, Spinal cord grafting

TOPIC: EPITHELIAL_GUT

606

SALIVARY GLAND ORGANOIDS AS THERAPEUTIC MODELS FOR RADIATION-INDUCED XEROSTOMIA

Jeon, Seong Gyeong - *Microbiology, CHA University of Life Sciences, Seongnam, Korea*
Choi, Woo Hee - *Microbiology, CHA University, Seongnam, Korea*
Bae, Dong Hyuck - *Microbiology, CHA University, Seongnam, Korea*
Lee, Jaeseon - *Regenerative Medicine R&D Center, Organoidsciences, Seongnam, Korea*
Seo, Jaehwi - *Regenerative Medicine R&D Center, Organoidsciences, Seongnam, Korea*
Lim, Young Chang - *Otorhinolaryngology-Head and Neck Surgery, Konkuk University School of Medicine, Seoul, Korea*
Yoo, Jongman - *Microbiology, CHA University, School of Medicine, Seongnam, Korea*

Abstract: Xerostomia is a symptom in which a patient's mouth is dry all the time due to a lack of saliva, lowering their quality of life. Pilocarpine is a parasympathomimetic agent that has resulted in significant improvement in symptoms of dry mouth. However, it has only a minor effect in patients who have lost all their salivary gland function. Adult stem cells have already been shown to have regenerative effects in organs such as the intestine and the heart, but their effect on the salivary glands is unknown. Here, we prove the potential effect of tissue regeneration capacity of the salivary gland organoids (SGOs) in the mouse model of radiation-induced xerostomia. The SGOs were able to self-renew and differentiate into major salivary lineages. Immunofluorescence staining analysis showing the expression of acinar cell marker AQP5, duct cell marker CK19. Furthermore, stimulation with the carbachol on SGOs increased the intracellular calcium gradient which is known to promote salivation. To assess SGOs function in vivo, we transplanted the GFP-expressing SGOs into the salivary gland of a mouse model of radiation-induced xerostomia. Engrafted GFP-expressing SGOs were observed in mouse salivary glands after 12weeks. SGOs have restored saliva production and significantly enhanced the regenerative potential of irradiated salivary glands. Together, this paper illustrates the potential of SGOs to regenerate the salivary gland and will pave the way for the regenerative therapy of salivary gland dysfunction.

Funding Source: 3D-TissueChip Based Drug Discovery Platform Program through the Korea Evaluation Institute of Industrial Technology funded by the Ministry of Commerce, Industry and Energy (20009773)
Keywords: salivary gland organoid, regenerative medicine, xerostomia

608

INTESTINAL ORGANOIDS: A TOOL TO UNDERSTAND GUT REPAIR FOLLOWING CONVENTIONAL AND FLASH RADIATION

Klett, Katarina - *Stem Cell Biology and Regenerative Medicine, Stanford University, Menlo Park, CA, USA*
Martin-Villa, Briana - *Bioengineering, Stanford University, Stanford, CA, USA*
Villarreal, Victoria - *Materials Science and Engineering, Stanford University, Stanford, CA, USA*
Melemenidis, Stavros - *Radiation Oncology, Stanford University, Stanford, CA, USA*
Viswanathan, Vignesh - *Radiation Oncology, Stanford University, Stanford, CA, USA*
Manjappa, Rakesh - *Radiation Oncology, Stanford University, Stanford, CA, USA*
Loo, Billy - *Radiation Oncology, Stanford University, Stanford, CA, USA*
Rankin, Erinn - *Radiation Oncology, Stanford University, Stanford, CA, USA*
Heilshorn, Sarah - *Materials Science and Engineering, Stanford University, Stanford, CA, USA*

Abstract: Although effective for ablating cancer cells, radiation therapy also damages healthy cells, including adult intestinal stem cells. This results in greatly reducing patient quality of life. An alternative method of delivering radiation known as FLASH (over ultra-short time periods, i.e., a fraction of a second rather than minutes) has demonstrated an ability to target cancer cells as effectively as conventional dose rate radiation, while preserving healthy tissue in murine models, although efficacy in humans is unknown. Enteroids are three-dimensional colonies of intestinal cells that mimic native tissue. These structures provide a powerful tool to study gut repair post conventional dose rate and FLASH radiation and explore the cellular mechanisms driving the observed effect. Murine and human enteroids were irradiated with varying doses of conventional dose rate and FLASH radiation (ranging from 2 Gy to 8 Gy) and imaged to quantify growth over time. At 3 Gy and 4 Gy doses respectively, murine and human enteroids receiving FLASH radiation grew statistically larger in size than those receiving conventional dose rate radiation. In addition to these changes in enteroid growth patterns, both murine and human enteroids had clear morphological differences dependent on whether they received no radiation, conventional dose rate radiation, or FLASH radiation. Current work is focused on analyzing gene expression changes post-radiation as well as utilizing Lgr5-GFP enteroids to investigate how FLASH radiation impacts intestinal stem cells.

Funding Source: NIH R01 EB027666, NIH R01 EB027171, NSF CBET 2033302, National Science Foundation Graduate Research Fellowship, Stanford Bioengineering and Vice Provost for Undergraduate Education Research
Keywords: Organoid, Radiation, Intestine

TOPIC: EYE AND RETINA

612

UNCOVERING THE ROLE OF MSX1 IN BIPOTENT RETINAL PROGENITOR CELLS OF THE CILIARY MARGIN

Abbasi, Sepideh - *Experimental Medicine (McGill), Center for Genetic and Neurological Diseases (IRCM), McGill University & Institut de Recherches Cliniques de Montréal (IRCM), Montréal, QC, Canada*
Boulan, Benoit - *Experimental Medicine (McGill), Cellular Neurobiology (IRCM), McGill University & IRCM, Montreal, QC, Canada*
Cayouette, Michel - *Anatomy and Cell Biology (McGill University), Medicine (Université de Montréal), Cellular Neurobiology Research Unit (IRCM), McGill University, Université de Montréal, IRCM, Montreal, QC, Canada*

Abstract: In the developing vertebrate retina, multipotent retinal progenitors are the main source of neurons and glia cells that build the mature retina. Interestingly, lineage tracing studies in our laboratory have identified a population of bipotent Msx1-positive (Msx1+ve) progenitors in the peripheral domain of the retina (ciliary margin, CM), which can produce non-neuronal cell types of the iris and ciliary body in addition to neurons of the peripheral retina. The neurogenic capacity of these Msx1+ve progenitors is restricted to embryonic stages, but the mechanisms regulating this competence have yet to be determined. Our objective is to determine these underlying mechanisms and investigate how their dysregulation affects retinal development and function. We hypothesize that Msx1 confers non-neurogenic potential to CM progenitors. To investigate this, we have generated a Msx1:CreERT2/flox; RosaYFP and αPax6Cre; RYFP; Msx1fl/fl mouse models, allowing the genetic deletion of Msx1 upon tamoxifen administration (in the CreERT2/flox mice) or upon embryonic expression of αPax6 at various developmental stages as well as simultaneous tracing of the Msx1 knockout lineage. To determine whether Msx1 expression is sufficient to confer non-neurogenic potential, we have overexpressed Msx1 in neurogenic progenitors of the central retina at postnatal day 0 and evaluated the ratio of neuronal fate at P7 and adult retina. Our preliminary analysis suggests that Msx1 is not sufficient to affect the rate of neurogenesis or modify the structure of the retina ($p>0.5$). Finally, transcriptomic analysis using single-cell RNA sequencing will determine whether the Msx1+ve population is heterogeneous, potentially containing sub-populations of neurogenic and non-neurogenic progenitors. Hence, YFP+ve cells were isolated from adult αPax6:Cre; RosaYFP; Msx1WT and αPax6:Cre; RosaYFP; Msx1fl/fl mice. Our preliminary analysis indicates that the Msx1fl/fl population contains a higher percentage of Mülar Glia, Rod, bipolar and amacrine subtypes. These results will shed light on the mechanisms by which Msx1+ve cells adopt a neurogenic vs. non-neurogenic fate. This knowledge will help reprogram endogenous non-neuronal cells into neurogenic progenitors which could be utilized to regenerate the retina following disease or injury.

Funding Source: Fonds de Recherche du Québec - Société et Culture (FRQSC) Canadian Institutes of Health Research (CIHR)
Keywords: Retina, Ciliary Margin, Msx1

TOPIC: LIVER

618

SOMATIC MOSAICISM REVEALS ADAPTIVE PATHWAYS IN CHRONIC LIVER DISEASE

Wang, Zixi - *Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, TX, USA*

Abstract: Lipid accumulation is detrimental to metabolic health on the organismal level, however it is unknown if somatic mutations that mitigate "cellular obesity" can increase competitiveness of regenerating cells within diseased tissues. To assess the biological impact of somatic mutations identified in human metabolic disease, we performed lineage tracing of mosaic clones in mice subjected to non-alcoholic steatohepatitis (NASH) inducing diets. Proof of concept studies with mosaic loss of Mboat7, a membrane-bound lipid acyltransferase identified through GWAS, revealed that increased steatosis can accelerate clonal disappearance in fatty livers. This result encouraged us to develop CRISPR mice with widespread somatic mosaicism in 63 well-known NAFLD genes. Aging of these somatically mosaic mice under normal or obesogenic diets allowed us to evaluate the fate of mutant clones in a side-by-side fashion in the liver. This in vivo competition assay revealed positive selection for somatic mutations that suppress lipogenesis, including several of the mutant genes recently identified in human NASH tissues. To prioritize additional candidate NASH genes, we screened 472 transcription factors and epigenetic regulators that are dysregulated on the mRNA level in human NASH livers. This effort identified somatic mutated genes that have strong influences on clonal expansion. In validation studies, liver-wide deletion of the top targets resulted in protection against NASH on the organismal level. Altogether, we show that positive selection for clonal fitness in mouse and human livers can identify pathways that regulate metabolic disease.

Keywords: Somatic mutations, NASH, Cell competition

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GENERATION OF HEPATOCYTE ORGANOIDS FROM MINIPIG LIVER

Ock, Sun A. - *Animal Biotechnology Division, National Institute of Animal Science (NIAS), RDA, Wanju-gun, Korea*
Kim, Seo-Yeon - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*
Jeong, Jiyeon - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*
Wi, Ha-Yeon - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*
Kim, Yeong-im - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*
Lee, Bo-ram - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*
Lee, Poongyeon - *Animal Biotechnology Division, National Institute of Animal Science, Wanju, Korea*

Abstract: There is an increasing number of patients with liver failure due to chronic diseases and infections. Liver transplantation is an important treatment option, but available organs are limited. Therefore, studies are being conducted on animals, for example pigs, with similar anatomical and physiological functions. To develop a therapy for liver failure, the present study produced liver subunits from hepatic organoids in a minipig model. Hepatocytes were collected from the liver of a 1-month-old male mini-pig using two step enzyme digestion and stored in liquid nitrogen. The thawed hepatocytes were seeded at 20,000 per 50 µL Matrigel dome (3D) and cultured in the HepatiCult organoid growth medium for 14 days (passage 0). As a control, the same number of hepatocytes were cultured on a 35 mm dish (2D) under the same culture conditions. Hepatocyte organoids were clearly observed from day 5 and their diameter was approximately 200 µm on day 14. These organoids were split with enzyme and sub-cultured at a ratio of 1:2 per well. The 2D culture of hepatocytes was performed similarly. We used qRT-PCR to analyze the expression of liver function genes (ALB, AAT, AFP, TF, etc.) in the groups cultured under 2D and 3D conditions. Compared to the 2D culture condition, the 3D condition was effective in maintaining liver function, especially ALB, AFP, and CYP3A29 expression. Under the 3D culture condition, hepatic organoids grew for more than 60 days, but under 2D culture conditions, hepatocytes stopped growing within a month. It was confirmed that the 3D environment not only prevented the decline of hepatocyte function, but also extended their proliferative period. The present study demonstrated the

potential of hepatocyte organoids for developing artificial liver support systems and novel drug tests.

Funding Source: This work was supported by the grant number PJ 015872.
Keywords: Porcine, Hepatocyte organoid, 3D culture

TOPIC: MUSCULOSKELETAL

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EXPLORATION OF CONDITIONS TO ENGINEER PRE-VASCULARIZED BONE USING TWO STEM CELL SOURCES AND ENDOTHELIAL CELLS

Luna, Katia M. - *Biology/BSCRIP/CIRM, San Diego State University, Chula Vista, CA, USA*
Grogan, Shawn - *Shiley Center for Orthopaedic Research and Education, Scripps Health, San Diego, CA, USA*

Abstract: Either human infrapatellar fat pad stem cells (IPFP-MSC) or human embryonic-derived MSC (ES-MSC) will be co-cultured alone with human umbilical vein endothelial cells (HUVECS) as microspheroids (MS) in either high density cultured only or seeded within a 3D printable fibrin-nanocellulose based gel (FiNC) to produce engineered pre-vascularized bone-like tissues. Conditions that promote neo-bone and prevascularized neo-bone in bioprintable FiNC gels will be useful for bioprinting bone tissues. The formation of pre-vascularized engineered tissue constructs with fused MS from either IPFP-MSC or ES-MSC with HUVECS will be useful as future implants into bone defects alone or in combination with osteochondral repair strategies.

Funding Source: CIRM
Keywords: neo-bone, IPFP-MSC, xeno-free ESC

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ANALYZING THE REGENERATIVE ROLE OF CDKN1A/P21 DURING MECHANICAL DISUSE WITH SINGLE CELL RESOLUTION

Kubik, Angela - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*
Cheng-Campbell, Margareth - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*
Allen, Noah - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*
Pendyala, Meghana - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*
Bai, Yang - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*
Chan, Deva - *Biomedical Engineering, Purdue University, West Lafayette, IN, USA*
Blaber, Elizabeth - *Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA*

Abstract: The degenerative consequences of prolonged mechanical unloading are of concern for civilian presence in space and Earth-based prolonged disuse. Previous research has revealed decreased regenerative capacity in bone marrow stem cells (BMSC) during spaceflight and subsequently defined an upregulation of CDKN1a/p21, a critical senescence mediator, in stem cell progenitors. We hypothesized that this may be due to aberrant mechanoregulatory signaling and an accumulation of reactive oxygen species (ROS), similar to that seen in degenerative aging models, thus defining p21 expression as a possible driver of the age-like regenerative decline seen in space. In this study, we aimed to explore the role of CDKN1a/p21 in in-vivo load-mediated stem cell-based tissue regeneration to further our understanding of the widespread regenerative dysfunction during altered loading paradigms. A 30-day hindlimb unloading (HU) study was performed on 16-week-old wildtype (WT) and CDKN1a/p21-/- (KO) mice. A subset of mice completed a treadmill-facilitated exercise regimen and/or a 14-day recovery period. Control groups were not unloaded, exercised, or recovered. Serum was separated from blood for detection of stress and bone resorption markers via ELISAs. Bone marrow was isolated, and single cell sequencing libraries were constructed and sequenced on the NextSeq550. Raw FastQ files were processed through the CellRanger pipeline, and the R toolkit Seurat was used for integrative pseudotime trajectory analysis and differential gene expression. Data reveals consistent systemic corticosterone levels for all conditions. Single cell RNA sequencing data shows shifts in immune progenitor populations and trajectories, specifically higher proportions of B cell progenitors in exercised HU groups. Additionally, hematopoietic progenitor lineages respond more sensitively to exercise-facilitated loading in KO groups compared to WT, suggesting that age-related stem cell-based immune dysregulations seen in microgravity may be mitigated with exercise and compounded in the absence of p21. These results demonstrate the beneficial effect of exercise in restoring the immune cell populations in the bone marrow following disuse and highlight the role of CDKN1a/p21 in suppressing regeneration during disuse.

Funding Source: This work is supported by NASA Space Biology Grant NNH14ZTT001N-0062 to Dr. Elizabeth Blaber and by New York Space Grant to Ms. Angela Kubik.
Keywords: space biology, stem cell regeneration, immune system

TOPIC: NEURAL

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THE APPLICATION OF BRAIN ORGANOID FOR DEVELOPMENTAL METABOLISM

So, Kyoung-Ha - *Institute of Chemical Process (ICP), Seoul National University, Seoul, Korea*
Song, WonMoon - *School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea*
Seok, HoDong - *College of Medicine, Seoul National University, Seoul, Korea*
Jeong, Haeln - *School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea*
Rajendran, Arun Kumar - *Institute of Chemical Process (ICP), Seoul National University, Seoul, Korea*
Hwang, Nathaniel S. - *School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea*

Abstract: Brain organoid has a lot of application for Bio-Health field, from basic science to regenerative medicine. We focused on metabolic synthesis during brain development using brain organoids. First, Human induced pluripotent stem cells (hiPSCs) were used source of Brain organoids that has induced with neuronal morphogens including ROCK inhibitor, bFGF, and extracellular matrix (ECM). Neural epithelium has matured Brain organoids with different composition of media and ECM for the evaluation of nutrient and scaffold effect (control/D, group/S). From this growth, Brain organoid has not difference than control group that means neuronal nutrient were supported brain organoid de-

velopment. Second, Brain organoid were developed from GBM patient derived hiPSCs and compared with normal hiPSCs. After over a month, this brain organoid size was little small then normal group that seems like growth retardation, whereas neuronal markers were detected such as SOX2, Tuj1, PAX6 in the immunohistochemical analysis. Third, Glioblastoma multiforme (GBM) cell line and 3D spheroids were analysis between the other culture condition. In this result, metabolic related pathway genes were expressed as like FABP3 (Fatty acid transporter), LIPE (Triglyceride metabolism), and CTP2 (Mitochondrial fatty acid oxidation disorders). In conclusion, we provide evidence that Brain organoid culture condition has available for various application and related with metabolic synthesis.

Funding Source: This research was supported by the National Research Foundation of Korea (NRF) [2019R1I1A1A01059554, 2020H1D3A1A04081286], and by the Ministry of Trade, Industry and Energy by the Korea government (No.20016553).
Keywords: Organoids, Brain, Metabolism

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DECODING THE PITUITARY'S STEM CELL BIOLOGY ACROSS LIFE

Laporte, Emma - *Development and Regeneration, Katholieke Universiteit Leuven, Belgium*
Vennekens, Annelies - *Development and Regeneration, KU Leuven, Belgium*
Hermans, Florian - *Development and Regeneration, KU Leuven, Belgium*
Vankelecom, Hugo - *Development and Regeneration, KU Leuven, Belgium*

Abstract: The endocrine pituitary gland dynamically remodels during key timepoints of life to meet the prevailing hormonal demands of the organism. Mechanisms underlying this plasticity remain poorly understood. In particular, it is not clear how the pituitary stem cells play a role and biologically behave during pituitary remodeling processes. In our studies, we aimed at decoding pituitary's stem cell biology and role during the neonatal vivid maturation phase of the gland, upon local injury in the gland and during its functional decline at aging, all by using state-of-the-art single-cell transcriptomics, transgenic mice and pituitary-derived organoid models. Single-cell RNA-sequencing (scRNA-seq) analysis exposed a pronounced WNT landscape in the neonatal gland, further shown to be involved in the high activation modus of its stem cells at that age. Transgenically inflicted pituitary damage, although not extra activating the neonatal stem cells, was found to be efficiently and fully restored. In contrast, injury in the adult gland promptly activates the pituitary stem cell compartment into higher proliferative activity and stemness pathways upregulation. The damage was found to be repaired after a couple of months although not fully. scRNA-seq interrogation led to the identification of interleukin-6 (IL-6) as pituitary stem cell activator in the adult gland, being upregulated after damage. At further aging, the pituitary's regenerative capacity completely disappeared, coinciding with the absence of stem cell activation following local injury as well as of the IL-6 upsurge, attributed to already high levels in the older gland and a pronounced inflammatory stem cell phenotype, all pointing to inflammaging as occurring in the whole organism at aging. Taken together, our studies provide deeper insight into pituitary's stem cell biology at key timepoints of life. A mouse pituitary single-cell atlas across life is being composed that will be instrumental to identify pathways and factors regulating the stem cells' behavior in the remodeling gland, which may eventually translate into restorative and rejuvenative strategies when pituitary function becomes compromised by damage or age.

Keywords: pituitary stem cells, organoids, single-cell RNA-sequencing

TOPIC: PANCREAS

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EFFICIENT GENERATION OF GP2+ MULTIPOTENT PANCREATIC CELLS THROUGH ANTERIOR DEFINITIVE ENDODERM

Juksar, Juri - *Nephrology, Leiden University Medical Center, Utrecht, Netherlands*
Lo Nigro, Antonio - *Hubrecht Institute, Utrecht, Netherlands*
Karanatsiou, Pagona-Maria - *Hubrecht Institute, Utrecht, Netherlands*
Paini, Diego - *Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Hanegraaf, Maaike - *Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Carlotti, Francoise - *Nephrology, Leiden University Medical Center, Leiden, Netherlands*
de Koning, Eelco - *Nephrology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Type 1 Diabetes is characterized by loss of insulin-producing β-cells in the pancreas leading to loss of glucose homeostasis. Cellular replacement therapies using human pluripotent stem cells (hPSC) are being developed to restore a functional β-cell mass. Recently, it was shown that hPSC-derived GP2+ multipotent pancreatic progenitor cells (MPC) generate β-like cells at higher efficiency compared to GP2- cells. Our goal was to create a differentiation protocol with a high efficiency of GP2+ cells using a step-wise approach following the first 3 stages of MPC differentiation – definitive endoderm (DE; S1), posterior foregut (PF; S2), MPC (S3). In S1, we aimed for high efficiency of anterior DE in 4 ESC and 2 iPSC lines. We compared the early DE stage of a protocol by Pagliuca, et al 2014 and Loh, et al 2014. The latter protocol lead to higher yield of anterior definitive endoderm (70-90% CD177+/CXCR4+) and single-cell RNA sequencing revealed early PF gene expression (HOXA1, FOXA1) at day 3. In PF development, FGF signalling is shown to affect pancreas and liver specification. We then optimised S2 of our differentiation protocol by inhibiting FGF signalling (MEK/ERK inhibitor PD0325901) leading to a higher efficiency of pancreatic progenitors (up to 95% PDX1+/GATA4+ (PD0325901) vs to 52% (FGF7)) and a significant increase in expression of dorsal pancreatic genes (MNX1, DLL1, FRZB). Additionally, PD0325901 treatment led to a significantly higher percentage of GP2+ cells at end of S3 (up to 51% GP2+ (PD0325901) vs 3% (FGF7)). Interestingly, inhibiting the upstream FGF receptor signalling directly (PD173074), led to a comparable outcome to MEK/ERK inhibition. However, inhibition of FGF signalling through AKT (AT7867) did not lead to similar outcomes (7% GP2+), suggesting that FGF inhibits MPC formation through the MEK/ERK pathway. In S3, our goal was to optimise the yield of GP2+ MPCs at day 11. By using T3, TPB, EGF and inhibiting BMP, ALK5, and Sonic Hedgehog we were able to achieve 60-90% GP2+ MPCs (6 cell lines). TPB significantly reduced premature NGN3+ cells (no TPB 8,4%, +TPB 1.7% of NGN3+ cells). In conclusion, by using a

step-wise approach in multiple PSC lines, highly efficient generation of GP2+ MPC could be achieved.

Funding Source: RegMedXB
Keywords: Pancreas, Beta-cell differentiation, Diabetes

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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CHARACTERIZATION OF VARIOUS CYTOKINES THAT MEDIATE THE THERAPEUTIC PARACRINE EFFECT OF UMBILICAL CORD-MSCS ON HUMAN OVARIAN FUNCTION

Seok, Jin - *Obstetrics and Gynecology, University of Chicago, IL, USA*
Park, Hang-Soo - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Liakath Ali, Farzana - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Mousaei, Mohammad - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*
Al-hendy, Ayman - *Department of Obstetrics and Gynecology, University of Chicago, IL, USA*

Abstract: Ovaries are the primary reproductive organ in women, the dysfunction of which results in serious disorders such as premature ovarian insufficiency (POI) and polycystic ovarian syndrome (PCOS). So far, although hormone replace therapy (HRT) is used for symptomatic relief in these patients, but limitations exist regarding appropriate treatment timing and patient age as well as side effects (e.g., breast cancer, thromboembolic events, and heart diseases). Hence, human mesenchymal stem cells (MSCs) have been reported their higher therapeutic potentials on degenerative disease through paracrine activities by cell-to-cell communication. As well as, they have been proven to have an excellent improvement effect on the ovarian dysfunction in women. However, this is only their mode of action (MOA), and detailed mechanism studies of stem cells on it are still lacking. Therefore, the objectives of this study are to analyze the therapeutic mechanism of MSCs for improving ovarian function. In order to select MSCs with high therapeutic effects, we performed the cytokine array using cell condition media from other origin stem cells. As a results, we were able to classify MSCs derived cytokines into various categories (e.g., angiogenesis, inflammation, immune response and etc.), and confirmed that UC-MSCs had higher potential than origin MSCs. Among them, it was confirmed that UC-MSCs secretes cytokines involved in migration and invasion activities (e.g., CCR7, CCR9, CCL27, CXCL11, CXCL14, CXCR1 and CXCR2). Based on these results, we confirmed that the mRNA expression related to migration (e.g., ALCAM, ICAM, VCAM, VLA4, E-cadherin, N-cadherin, and P-cadherin) increased in UC-MSCs than other MSCs. Especially, the expression of active MMP-2 and MMP-9 related to regulation of extracellular matrix degradation as well as the higher activities of invasion and migration showed higher on 48h and 72h. Our study demonstrates the higher engraftment potentials of UC-MSCs for the treatment of ovarian dysfunction diseases and our findings are important in identifying mechanism for the treatment of ovarian dysfunction using stem cells. Taken together, the findings offer new insights into further understanding of stem cell therapy for reproductive systems and should provide new avenues to develop more efficient therapies.

Funding Source: This study supported by start-up fund of the University of Chicago (AA).
Keywords: Mesenchymal stem cells, Paracrine effect, Stem cell therapy

TOPIC: PLURIPOTENT STEM CELLS

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CEREBROVASCULAR MODELING OF COCKAYNE SYNDROME B USING IPSC-DERIVED BRAIN ORGANOIDS AND VASCULAR CELLS

Staples-West, Aaliyah - *San Diego State University, San Diego State University/CIRM, San Diego, CA, USA*
Papes, Fabio - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Teng, Evan - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Muotri, Alysson - *Pediatrics, University of California, San Diego, La Jolla, CA, USA*
LaMontagne, Erin - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*
Engler, Adam - *Bioengineering, University of California, San Diego, La Jolla, CA, USA*

Abstract: Cockayne Syndrome B (CSB) is an autosomal recessive disorder characterized by neurodegeneration and premature aging. The ERCC6 gene is found in mice but mouse models fail to recapitulate all the neuronal and vascular defects of this disease. Thus, we are using in vitro methods using human cell lines to better understand the cellular defects that cause CSB. Our goal is to replicate the vascular defects in the frontal cortex of CSB patients using patient-derived induced pluripotent stem cells (iPSCs) to generate and characterize vascularized brain organoids. CSB iPSCs will be differentiated into vascular endothelial cells (vECs) and undergo a series of assays to characterize defects in vascular function. These vECs will then be implemented into vascularized brain organoids using a unique microfluidic system that has the capacity to mimic shear stresses caused by blood flow in the brain and reveal further functional defects in CSB vECs. To ensure that cellular and molecular defects are caused by the mutations in ERCC6 rather than other genetic or epigenetic alterations in patient-derived cells, we will generate isogenic iPSC lines harboring ERCC6 mutations and characterizing their differentiated vECs alongside our CSB patient cells. I hypothesize that these experiments will reveal defects in vEC structure and function due to the presence of ERCC6 mutations that may contribute to the cerebrovascular pathophysiologies seen in CSB patients. Modeling the cerebrovascular pathways of CSB will elucidate the origins of CSB at the cellular level and potentially reveal insight that may contribute to new therapies against this disease.

Keywords: Brain Organoids, Vascular Cells, Cockayne Syndrome B

TOPIC: EARLY EMBRYO

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HYDROGEL ENVIRONMENT INDUCES IBLASTOID GENERATION DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Imamura, Satoshi - *Kyoto University Institute for Advanced Study, Kyoto University, Kyoto-shi, Japan*
Wen, Xiaopeng - *College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Japan*
Terada, Shiho - *institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan*
Yoshimoto, Koki - *Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan*
Kamei, Ken-ichiro - *Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan*

Abstract: A blastocyst will not only help study the early development of a cell but can also be used in drug discovery for pregnancy failure and birth deficiency prevention. However, especially in human beings, it is very difficult to obtain blastocysts from fertilized eggs for fundamental research as well as industrial usage due to limited cell sources / donors and ethical concerns. Recent studies have reported methods of creating blastocysts using human pluripotent stem cells (hPSCs). However, most of those studies use cells that have been transformed into naïve cells from primed hPSCs. Here, we develop a method to generate iBlastoids from primed hPSCs in a three-dimensional (3D) hydrogel environment. We name it as the HG-iBlastoid. This method uses a thermo-responsive hydrogel [a copolymer of poly (N-isopropylacrylamide) and poly (ethylene glycol) (PNIPAAm-PEG)], which provides cultured hPSC aggregates with mild physical stimuli. Importantly, the hydrogel needs not to adhere the cells to prevent migration into the gel. Additionally, because it can perform a sol–gel transition via temperature, the hydrogel allows indroducing and harvesting of HG-iBlastoids from the solution at a low temperature < 20 °C), and culture them at 37 °C. To visualize the expression of octamer-binding transcription factor 4 (OCT4) ICM marker, we used OCT4 promoter-driven KhES1 hESCs with an enhanced green fluorescent protein (K1-OCT4-EGFP). Using K1-OCT-EGFP, we cultured with DMEM supplemented with 10% FBS with the hydrogel for four days, and with iBlastoid medium with the hydrogel for four days. To confirm the cellular distribution of trophoblasts and ICM cells in HG-iBlastoids, we observed the expression patterns of OCT4 and CDX2 in HG-iBlastoids via whole-mount immunocytochemistry. In conclusion, we established a simple method to generate human HG-iBlastoids from only hPSCs. The HG enables better environments for the formation of HG-iBlastoids, which suggests that a certain physical property of the gel plays an important role in the blastocyst formation and not the fluid property.

Funding Source: The Japan Society for the Promotion of Science (JSPS;17H02083).
Keywords: Blastoid, Hydrogel, hESC

TOPIC: EPITHELIAL_LUNG

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USING INSIGHTS FROM THE FIRST HUMAN FETAL SALIVARY GLAND SINGLE-CELL ATLAS TO INFORM HUMAN IPSC-DERIVED SALIVARY GLAND ORGANOID DEVELOPMENT

Ehnes, Devon D. - *Institute for Stem Cells and Regenerative Medicine, University of Washington, Bothell, WA, USA*
Alghadeer, Ammar - *Department of Biomedical Dental Sciences, Imam Abdulrahman bin Faisal University, Seattle, WA, USA*
Hanson-Drury, Sesha - *Department of Oral Health Sciences, University of Washington, Seattle, WA, USA*
Zhao, Yan-Ting - *Department of Oral Health Sciences, University of Washington, Seattle, WA, USA*
Jung, Hee Yun - *Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA*
Tilmes, Gwen - *Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA*
Mathieu, Julie - *Institute for Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA*
Ruohola-Baker, Hannele - *Department of Biochemistry, University of Washington, Seattle, WA, USA*

Abstract: Multiple pathologies and traumas can disrupt salivary gland function, but there do not yet exist definitive therapeutics or regenerative approaches to address salivary gland loss, likely due to significant gaps in our understanding of salivary gland development. Moreover, studies have identified the salivary epithelium as a major player in COVID-19 infection and spread, necessitating an organoid that can fill both clinical and research needs. We conducted single-cell sequencing on human fetal salivary gland tissue from multiple developmental stages and constructed a developmental atlas. Our single-cell analyses have revealed developmental branchpoints, a novel stem cell-like population, and key signaling pathways in the human developing salivary gland. Trajectory and transcriptional analysis suggest that earliest progenitors yield excretory duct and myoepithelial cells, as well as a transitional population that will yield later ductal cell types. Importantly, this analysis revealed a previously undescribed population of stem cell-like cells that are derived from striated duct and expresses high levels of genes associated with stem cell-like function. We have observed these rare cells not in a single niche location but dispersed within the developing duct at later developmental stages. Our data also suggests that ductal progenitors that give rise to intercalated and striated duct are more developmentally akin to intercalated duct, suggesting that the suppression of the striated duct gene program yields intercalated duct. We also observe temporally distinct populations of ACTA2+ myoepithelial cells, one a transient population that seems to be involved in early tissue morphogenesis, and a later functional one surrounding the ducts. Finally, we have uncovered a previously undescribed role of FOS/JUN (AP-1) signaling in driving distal tip reorganization to yield acinar precursors. Early organoid development efforts suggest that we can use the information acquired here to drive cells to self-organize into 3D duct-like structures with lumens that then branch outward, expressing KRT19 that marks duct tissue during human fetal salivary gland development. Our work lays the groundwork for the development of translational human therapeutics and ongoing developmental studies.

Keywords: Exocrine organoid, Salivary gland, Regeneration

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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THE IMPACTS OF POLICY ON RESEARCH WITH HUMAN EMBRYONIC STEM CELLS ON COMPOSITION OF STEM CELLS FOR CARDIAC RESEARCH

Kishi, Naoko - *Graduate School of International Social Sciences, Yokohama National University, Yokohama, Japan*

Abstract: To investigate the impact of political interventions on stem cell research, this study analyzes the effect of the hESC prohibitive policy on scientists' choices of stem cells for cardiac research. It hypothesizes that political prohibition on hESC would drive scientists to choose stem cells other than ESC. In other words, it assumes that scientists may avoid ESC and choose the other stem cells because political obstacles for hESC would make them aware of its difficulty in ESC research's future development. The analysis exploits the variation in the location of the first authors' affiliations to identify the impact of the political intervention on scientists' stem cell choices for research. The dataset is constructed from published articles collected using PubMed search and includes studies on ESC, iPSC, MSC, and BMC for all species' hearts. The sample articles were publications from 2009 to 2019. An observation is the choice of stem cells for cardiac research in an individual article. The analysis uses probit regression. The dependent variable is whether the chosen stem cell for research is ESC or not. Furthermore, this study investigates whether scientists choose iPSC and MSC/BMC to study cardiac research. The independent variable is whether countries in which affiliated organizations of first authors are located adopt a policy that prohibits research using human embryo. Control variables include dummy variables about publication year of articles and top ten countries in the number of sample articles. The countries in dummy variables include Canada, China, France, Germany, Italy, Japan, South Korea, the Netherlands, the United Kingdom, and the United States. The results demonstrate that countries' prohibitive policies decreased the number of articles that used ESC. In conclusion, the prohibitive policies bias the composition of stem cells chosen for cardiac research. The data also demonstrate the variations of stem cell compositions across countries. For example, scientists in Japan are likely to choose iPSC, and scientists in China are likely to be involved in MSC/BMC research. The limitation of this study is that the impacts of other factors, including the amount of funding in respective stem cells on scientists' stem cell choices, are not considered.

Funding Source: This study was supported by Grants-in-Aid from the Japan Society for the Promotion of Science (Kiban-C-21K00247).
Keywords: Political interventions, Stem cells, Cardiac regeneration

TOPIC: IMMUNE SYSTEM

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THE THYMUS, A CARDIAC SURGERY MEDICAL WASTE AS A VALUABLE PROGENITOR/STEM CELL POOL

Antica, Mariastefania - *Molecular Biology, Ruder Boskovic Institute, Zagreb, Croatia*
Bódi, Ildikó - *Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary*
Matulić, Maja - *Division of Molecular Biology, Faculty of Science, Zagreb, Croatia*
Belina, Dražen - *Department of Cardiac Surgery, University Hospital Centre Zagreb, Croatia*
Heckel, Darko - *Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia*
Škrtić, Anita - *Pathology, Merkur Clinical Hospital, Zagreb, Croatia*
Prodan, Zsolt - *Kids Heart Center, György Gottsegen Hungarian Institute of Cardiology, Budapest., Hungary*
Grčević, Danka - *Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia*

Abstract: Congenital athymia or surgical thymectomy at early age in children with congenital heart diseases have a clear immunological impact. Children born with a complex heart developmental disorder need a life-saving heart surgery and due to the size and location of the thymus, just above the heart and the major blood vessels, the surgeon is forced to perform total thymectomy. It has been shown that there are long-term consequences of thymectomy in the functioning of the immune system like autoimmune processes, allergic reactions, or even cancer development. In order to protect the immune system in those patients we present here our research on thymic epithelial stem cell progenitors as the basis for human thymus regeneration. We applied immunohistochemistry and flow cytometry methods to find cells from the human thymus with stem cell characteristics. We were able to distinguish by antibody labelling of thymus sections very rare cells expressing NANOG, FoxN1 and Plet-1. By means of existing and modified enzymatic and negative selection methods we obtained enriched single cell suspensions of CD45negEpCAMneg cells and we show by flow cytometry and multiplex-PCR analysis further stem cell characteristics of a minor population of human thymic cells. Finally, we employed the in vitro 3D-cultures of the thymic cells in low attachment conditions in order to evaluate the formation of thymospheres as a measure of their self-renewal capacities. We use the spheres as the founding elements of reaggregated organotypic cultures together with CD34pos cells for T cell development and differentiation. In conclusion we present human thymic cells that have progenitor/stem cell features and that we believe can be further cultured or developed as the backbone of thymus regeneration studies in order to protect the immune system in congenital or iatrogenically caused thymic defects.

Funding Source: The Croatian Science Foundation Grant IP-2020-02-2431 The Terry FoxZagreb Run The Scientific Centre of Excellence for Reproductive and Regenerative Medicine (Grant Agreement KK01.1.1.01.0008 European Regional Development Fund)
Keywords: Stem cells from developed tissues, Thymus, Thymic epithelial progenitor/stem cells

TOPIC: MUSCULOSKELETAL

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IDENTIFYING FACTORS TO PROMOTE EX VIVO EXPANSION OF SKELETAL STEM/PROGENITOR CELLS FOR THE TREATMENT OF OSTEOPOROSIS

Ferreyra, Stephanie - *Biological Sciences, Sacramento State University, La Verne, CA, USA*
Le, Bryan - *Integrative Pathobiology, UC Davis, Sacramento, CA, USA*
Cressman, Amin - *Integrative Pathobiology, UC Davis, Sacramento, CA, USA*
Yao, Wei - *Musculoskeletal Health, UC Davis, Sacramento, CA, USA*
Lee, Mark - *Orthopaedic Surgery, UC Davis, Sacramento, CA, USA*
Fierro, Fernando - *Stem Cell Program, UC Davis, Sacramento, CA, USA*

Abstract: Osteoporosis is a silent condition characterized by loss of bone structure and strength. Current standard of care focuses on halting bone resorption, by targeting osteoclasts. However, this approach minimally promotes restoration of the lost bone density. Stem cell therapy is an attractive candidate for the treatment of osteoporosis by allowing for the remodeling of the skeletal system. Despite recent progress in understanding the biology of skeletal stem/progenitor cells (SSPCs) and their progeny, due to the low numbers of SSPCs and limited markers to identify them, potential therapies utilizing these cells have been challenging. We have isolated human CD45-CD51+CD200+ cells from bone marrow aspirates (hip) and from RIA (Reamer Irrigator Aspirator) surgical waste (effluent) during the preparation of autologous bone graft material (femur). Single cell RNA sequencing suggests that this cell compartment is homogenous and corresponds to SSPCs. Under standard culture methods, SSPCs become identical to bone marrow stromal cells/mesenchymal stem cells (MSCs). However, SSPCs kept ex vivo in the original RIA-effluent undergo a significant expansion during the first 24 hours without any alterations in immune phenotype. A combined proteomic analysis using four RIA effluents shows various candidates with roles in ossification and biomineralization that could favor SSPC expansion. Remarkably, after intravenous administration, the cells home efficiently to bone/bone marrow in sublethally irradiated NSG mice. Calcein staining shows that SSPCs strongly contribute to the newly formed bone, supporting the notion that SSPCs contribute to bone repair through direct differentiation into osteoblasts. As little as 1,200 SSPCs promote significant bone repair in a fracture model in immune deficient NSG mice. Finally, we show that SSPCs may promote bone restoration in ovariectomized NSG mice, supporting the notion of using SSPCs to treat systemic skeletal defects, such as osteoporosis. Altogether, our results suggest that CD45-CD51+CD200+ SSPCs could become important therapeutic targets for orthopedic regenerative medicine.

Keywords: Osteoporosis, Skeletal Stem Progenitor Cells, Reamer Irrigator Aspirator (RIA)

TOPIC: NEURAL

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DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS TOWARDS A NEURAL LINEAGE: IN VITRO STUDY

Hussain, Issam I. - *Life Sciences, University of Lincoln, UK*
Bownes, Emma - *Life Sciences, University of Lincoln, UK*
Rea, Carol - *Life Sciences, University of Lincoln, UK*

Abstract: Mesenchymal stem cells (MSCs) are a promising cell-based therapeutic intervention of neurodegenerative diseases, such as Alzheimer's disease and other neurological conditions, with the capacity for neurogenic differentiation. MSCs possess a broad regenerative potential and immuno-suppressive properties and provide neurotrophic support when applied to pathologies of the nervous system. Furthermore, MSCs can express characteristic neural markers, including neuroectodermal stem cell marker (Nestin) and a microtubule-associated protein 2 (MAP2) when cultured in specific culture conditions. The purpose of this study is to determine if MSCs can successfully transdifferentiate into neuron-like cells, evidenced by the expression of neural markers for a specific period and changes in cell morphology towards a neuronal phenotype. Bone-marrow-derived MSCs were cultured in a 5% CO2 incubator at 37°C in growth media, and the cell morphology of undifferentiated MSCs was observed. In addition, a proliferation assay was performed, and a trypan-blue dye-exclusion assay determined cell viability. MSCs were later induced with neurogenic differentiation media and incubated in a 5% CO2 incubator at 37°C over 24,48,72 and 96 hours. Quantitative and qualitative immunophenotyping of cells for specific neural markers was achieved using immunofluorescent staining of Nestin and MAP2 sandwich ELISA, respectively. MSCs demonstrated a high proliferative capacity and high cell viability in culture. In addition, BM-MSCs harness neurogenic potential, evidenced by altered cell morphology and characteristic neural markers. Under-exposed to neurogenic differentiation media, cells demonstrated positive expression for Nestin and MAP2 and adopted a neuronal phenotype with significantly higher at first 24 hours. Previous studies have documented that MSCs are a valuable source of neuron-like cells in neurological disease or neuronal injury cases. However, further research is required to validate these findings and potential therapeutic approaches for neurological diseases.

Keywords: Mesenchymal stem cells, Differentiation, Neural like cells

TOPIC: PLURIPOTENT STEM CELLS

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STUDY OF A NOVEL FORMULATION OF ULTRA-HIGH VISCOUS ALGINATE AND GELMA FOR ADHERENT CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Nenninger, Beate-Sophie - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering, Wuerzburg, Germany*
Gepp, Michael - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*
Park, Dongjeong - *Fraunhofer Project Center for Stem Cell*

Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany
Zimmermann, Heiko - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*
Neubauer, Julia - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*

Abstract: Hydrogels are commonly used as matrix for physiological cell based models for screenings, disease modelling and cytotoxicity screenings. There is a wide range of hydrogel materials, each with different properties and crosslinking principles. By default, the hydrogel material is used to enhance in vitro cultivation of a 2D cell culture by means of chemical signals or structural arrangement. The combination of two different hydrogels could provide an approach to vary, control, and modify the hydrogel properties and, in consequence, the cell-matrix-interface. This forms the basis for possible further application methods of hydrogels, such as 3D printing. In order to examine the potential of a particular hydrogel combination, fundamental cell culture parameters like cytotoxicity, adhesion and proliferation must be verified especially for sensitive cell lines such as human induced pluripotent stem cells (hiPSCs). In this work, an approach to overcome unphysiological conditions by implementing and characterizing a novel formulation of two hydrogels that exhibit different crosslinking methods was investigated. Ultra-high viscous alginates form hydrogels in the presence of divalent cations (e.g. Ba2+ or Ca2+) via ionotropic gelation. Gelatin-methacrylol (GelMA) is based on photo crosslinking and has highly modifiable mechanical properties. By combining these hydrogels, it is possible to generate surfaces with adjustable stiffness. As growth surface, our data indicates that the proliferation rate of hiPSCs was slightly decreased comparing to hiPSCs growing on standard petri dish, whereas at the same time the cell-matrix-contacts increased. In addition, we observed that endothelial cells build strong intercellular connections, it can be hypothesized that this will be advantageous for the functionality of hiPSC-derived cells e.g., cardiomyocytes. In conclusion, we developed a reliable method for the combination of ionic and photo crosslinking at the same time. To evolve (stem-) cell culture techniques our hydrogel surface provides a wide range of different stiffness and various mechanical properties, which can adapt to the respective application and cell types. Structural effects were observed during crosslinking what might be interesting for future cell model development.

Funding Source: This work was supported by the Bavarian Ministry of Economic Affairs, Regional Development and Energy.
Keywords: human induced pluripotent stem cells, alginate, GelMA

POSTER SESSION III: ODD

3:00 PM – 4:00 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

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THE MULTI-LINEAGE TRANSCRIPTION FACTOR ISL1 CONTROLS CARDIOMYOCYTE CELL FATE THROUGH INTERACTION WITH NKX2.5

Maven, Bonnie E. - *UCSF Graduate Division, The Gladstone Institutes, San Francisco, CA, USA*
Gifford, Casey - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Weilert, Melanie - *Pathology and Laboratory Medicine, Stowers Institute, Kansas City, MO, USA*
Gonzalez-Teran, Barbara - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Hüttenhain, Ruth - *Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA*
Pelonero, Angelo - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Ivey, Kathryn - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Samse-Knapp, Kaitlen - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Kwong, Wesley - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Gordon, David - *Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA*
McGregor, Michael - *Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA*
Nishino, Tomohiro - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Costa, Mauro - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Okorie, Eyuche - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Rossman, Sage - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Krogan, Nevan - *Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA*
Zeitlinger, Julia - *Pathology and Laboratory Medicine, Stowers Institute, Kansas City, MO, USA*
Srivastava, Deepak - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Congenital heart disease (CHD) is a leading cause of infant mortality in the US and is commonly thought to arise from perturbations of transcription factors (TFs) that guide lineage choices during cardiac development. ISLET1 (ISL1) is one such TF, although it also directs differentiation of other cell types, including motor neuron progenitors (MNPs) and pancreatic islet cells. Although cellular specificity of ISL1 function is likely achieved through combinatorial interactions, its essential cardiac interacting partners are unknown. By assaying ISL1 genomic occupancy in human iPSC-derived cardiac progenitors (CPs) or MNPs and

leveraging the deep learning approach BPNet, we identified cell-type-specific motifs of other TFs that predicted ISL1 occupancy in each lineage, with the NKX2.5 motif being most closely associated to ISL1 in CPs. We demonstrated ISL1 forms a protein complex with NKX2.5 in CPs, and the two regulated similar gene networks. NKX2.5 co-occupied nearly two-thirds of ISL1-bound loci, and ISL1 was dependent on NKX2.5 for CP-specific localization. Furthermore, overexpression of NKX2.5 in MNPs led to ISL1 redistribution to CP-specific loci. These results reveal how ISL1 can guide differential lineage choices through a combinatorial code that dictates genomic occupancy and transcription.

Funding Source: This research was funded in part with the Predoctoral Fellowship from the American Heart Association (19PRE34380715).
Keywords: cardiac development, combinatorial code, transcriptional regulation

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HIGHLY EFFICIENT GENERATION OF PACEMAKER-LIKE CELLS FROM INDUCED PLURIPOTENT STEM CELLS FOR FUNCTIONAL GENOMICS

Engel, James L. - *Cardiometabolic Disorders, Amgen, South San Francisco, CA, USA*
Lu, Daniel - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Zhang, Xianglong - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Arias, Vanessa - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Mohan, Rajiv - *Cardiology Division, University of California, San Francisco, CA, USA*
Tanasa, Bogdan - *ADI Drug Discovery, Amgen, South San Francisco, CA, USA*
Fernandez Vila, Olaia - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Hale, Christopher - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Li, Chi-Ming - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Hsu, Yi-Hsiang - *Genome Analysis Unit, Amgen, South San Francisco, CA, USA*
Vedantham, Vasanth - *Cardiology Division, Cardiovascular Research Institute, University of California, San Francisco, CA, USA*
Ang, Yen-Sin - *Cardiometabolic Disorders, Amgen, South San Francisco, CA, USA*

Abstract: Pacemaker cells within the sinoatrial node (SAN) have a distinct gene expression program that allows them to integrate input from the autonomic nervous system and fire automatically to initiate each heartbeat. Dysfunction of this neural-cardiac system is irreversible and leads to the development of symptomatic bradycardia, the most common reason for permanent pacemaker implantation. However, the gene regulatory mechanisms that underlie pacemaker cell specification and differentiation remain incompletely understood, partly due to the rarity of this cell type and the difficulty of isolating large numbers of pacemaker cells for molecular analysis. Here, we developed a high-yield, high-purity scalable monolayer protocol to generate pacemaker-like cardiomyocytes from human induced pluripotent stem cell (hiPSC) for studying the developmental, molecular, and functional aspects of SAN gene regulation. To gain insight into the genomic architecture of human pacemaker cells, we used this cellular model to perform single cell (sc) RNA-sequencing, sc-ATAC-sequencing and CUT&RUN-sequencing analyses for pacemaker cell enriched transcriptional regulators. Time course analyses of cellular differentiation and integration of epigenetic and expression datasets with human genetic data will be presented, with a goal of identifying novel pathways that will deepen our understanding of SAN development and disease in humans. Overall, this systems biology approach to dissect human pacemaker cell development establishes a new and powerful in-vitro model for genetic analysis of human heart rhythm and for development of therapeutics for disorders of cardiac pacemaking.

Keywords: hiPSC derived pacemaker cells, epigenetics of cardiac differentiation, sinoatrial node development

TOPIC: EARLY EMBRYO

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INTERLEUKIN-7 ENHANCES INNER CELL MASS BY REGULATING PI3K/AKT SIGNALING IN PORCINE PARTHENOTE EMBRYO DEVELOPMENT

Oh, Dongjin - *Veterinary Medicine, Chungbuk National University, Cheongju-si, Korea*
Hyun, Sang-Hwan - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Interleukin-7 (IL-7) has been identified as a crucial factor for cell development, proliferation, and survival. IL-7 plays a significant role in porcine oocyte maturation, but its role in embryonic development has not been explored. Here, we investigate the effects of IL-7 treatment on porcine embryos during in vitro culture (IVC) to determine whether it can increase embryonic development at the preimplantation stage. First, we found that IL-7 and its binding to IL-7Rα (IL-7R) were localized in porcine parthenote embryos. In parthenogenetic activation (PA), the cleavage rates were significantly higher in 10- and 100 ng/mL IL-7-treated group (83.4% and 78.4%) than in the control group (63.4%). Day 7 after PA, only the 10 ng/mL IL-7 treated group showed significantly higher blastocyst formation rates (71.3%) than the control group (51.9%). Furthermore, 1- and 10 ng/mL IL-7 treatment groups exhibited significantly increased intracellular GSH levels, and only 10 ng/ml IL-7 treatment group displayed significantly decreased intracellular ROS levels compared with the control group. Then, we examined the apoptosis rates of blastocysts by TUNEL assay. The 1- and 10 ng/mL IL-7 treatment groups (13.8% and 8.7%) displayed significantly decreased apoptosis rates of blastocyst compared with the control group (19.2%). Furthermore, we analyzed the gene expression in blastocysts treated with 10 ng/mL IL-7 using quantitative PCR (qPCR). BAX level was significantly decreased, and MCL1 level was significantly increased in IL-7-treated blastocyst compared to control. The IL-7 supplementation significantly improved IL-7 signaling-related genes, PIK3R1, AKT1, and ERK2, compared to control. OCT4 and NANOG levels were significantly increased in IL-7-treated blastocyst. Moreover, we demonstrated that supplementation with IL-7 enhanced the inner cell mass (ICM) marker SOX2+ cells, ICM ratio, and pAKT expression, improving blastocyst quality. In conclusion, for the first time, we proved the localization of IL-7 and IL-7R in porcine preimplantation embryos in vitro. Furthermore, we suggest that IL-7 supplementation enhances the embryonic development and ICM

ratio by regulating PI3K/AKT signaling during porcine PA embryo development in vitro.

Funding Source: This work was supported by grants from the "NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)" and "IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.
Keywords: Porcine embryos, Embryonic development, Interleukin-7

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DROPLET-BASED SINGLE-CELL TOTAL RNA-SEQ REVEALS DIFFERENTIAL NON-CODING EXPRESSION AND SPLICING PATTERNS DURING MOUSE DEVELOPMENT

Alemany, Anna - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Salmen, Fredrik - *van Oudenaarden Lab, Hubrecht institute, Utrecht, Netherlands*
De Jonghe, Joachim - *Department of Biochemistry, University of Cambridge, UK*
Kaminski, Tomasz - *Department of Biochemistry, University of Cambridge, UK*
Parada, Guillermo - *Wellcome Genome Campus, Wellcome Sanger Institute, Hinxton, UK*
Hemberg, Martin - *Wellcome Genome Campus, Wellcome Sanger Institute, Hinxton, UK*
Hollfelder, Florian - *Department of Biochemistry, University of Cambridge, UK*
van Oudenaarden, Alexander - *van Oudenaarden Lab, Hubrecht institute, Utrecht, Netherlands*

Abstract: In recent years, single-cell transcriptome sequencing has revolutionized biology, allowing for the unbiased characterization of cellular subpopulations. However, most methods amplify the termini of polyadenylated transcripts capturing only a small fraction of the total cellular transcriptome. This precludes the detection of many long non-coding, short non-coding and non-polyadenylated protein-coding transcripts. Additionally, most workflows do not sequence the full transcript hindering the analysis of alternative splicing. We therefore developed VASA-seq to detect the total transcriptome in single cells. VASA-seq is compatible with both plate-based formats and droplet microfluidics. We applied VASA-seq to over 30,000 single cells in the developing mouse embryo during gastrulation and early organogenesis. The dynamics of the total single-cell transcriptome result in the discovery of novel cell type markers many based on non-coding RNA, an in vivo cell cycle analysis and an improved RNA velocity characterization. Moreover, it provides the first comprehensive analysis of alternative splicing during mammalian development.

Funding Source: Dutch Research Council (NWO) - Talent Programme (Vi.Vidi.203.050) Novo Nordisk Foundation grant - reNEW (NNF21CC0073729)
Keywords: single cell RNA sequencing, embryonic development, RNA biotypes

TOPIC: EYE AND RETINA

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TRACING MOUSE EARLY RETINAL PROGENITOR CELLS IN OPTIC-CUP ORGANOIDS

Zabiegalov, Oleksandr - *Ophtalmology, University of Lausanne/ Eye Hospital Jules Gonin, Lausanne, Switzerland*
Arsenijevic, Yvan - *Ophthalmology, University of Lausanne, Switzerland*
Kostic, Corinne - *Ophthalmology, University of Lausanne, Switzerland*
Udry, Florian - *Ophtalmology, University of Lausanne, Switzerland*

Abstract: High number of people with severe visual impairments warrants searches for new models to develop therapies. Combined with the latest biotechnological approaches, retinal organoids (RO) become a powerful tool to delve deeper into retinal diseases causes and development. However, handful of imperfections precludes its broader use. One limitation is the heterogeneity of optic-cup organoid development synchrony, likely caused by interference with non-retinal tissues. To address this shortcoming we hypothesize that derivation of RO from population of early retinal progenitor cells, instead of pluripotent stem cells, will reduce RO formation heterogeneity. To have means for tracing and separating progenitor cells, we used CRISPR nickase gene editing system to create a reporter mouse embryonic stem cell line, in which expression of retinal progenitor-specific gene Rax (aka Rx) drives the expression of the fluorescent protein gene mCherry. For this modification we used Crx-GFP mouse embryonic stem cell line derived in the lab, which allows GFP tracing of nascent photoreceptors in RO. We expect that the mCherry-positive cell population contains retinal progenitors as well as cells forming the niche for the eye field induction. With help of CRISPR web toolbox CHOPCHOP, we designed vectors, transfected mouse stem cells, performed antibiotic selection, propagated single-cell clones and analyzed 5 lines with PCR and sequencing. One line had desired insert and was used to excise selection cassette by transfection with Cre recombinase carrying vesicles. Single-cell clones were derived and analyzed. Out of 15 clones used to differentiate to RO, only three clones could produce ultimately organoids. We showed that our new line possess errorless insertion of mCherry gene to one allele of mouse genome, and fluorescent protein is expressed during early and late development of retinal organoids. Immunohistochemistry analyzes of RO slices revealed appearance of mCherry-positive retinal progenitor cells starting from forth day after aggregation. We will further characterize the progenitor cells, confirm their identity and aggregate them to derive RO. This new cell line can serve in the future for retinal studies aiming at tracing retinal progenitors and mature rods to study their biology in different conditions.

Keywords: Organoid, Retina, Progenitor

TOPIC: HEMATOPOIETIC SYSTEM

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MUCINS, MICROVILLI AND MIGRATION: CONTROLLING ADHESION OF LEUKEMIC STEM CELLS IN FLOW

Aldehaiman, Mansour - *BESE, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Nozue, Shuho - *BESE, King Abdullah University Of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Habuchi, Satoshi - *BESE, King Abdullah University Of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Merzaban, Jasmeen - *BESE, King Abdullah University Of Science and Technology (KAUST), Thuwal, Saudi Arabia*

Abstract: Hematopoietic stem/progenitor cells (HSPC) migration and engraftment to the bone marrow is dependent on E-selectin expression on the bone marrow endothelium. Using a mass spectrometry (MS), molecular and biochemical approaches, we identified a repertoire of ligands that bind these selectins on HSPCs. Knockdown of ligands in HSPCs either individually or together provided insight into which ligands support slow rolling and adhesion on the endothelial selectins. Interestingly, knocking out CD34, an E- and P- selectin ligand identified in our lab on KG1a cells (HSC model cell line) and HSPCs, decreased the number of adhering cells binding E-selectin and increased the speed of cells in flow. Using real-time live cell imaging of these cells under shear flow, the long, thin, flexible structures protruding out from the rear (tethers) and the front (slings) sides of the cell as it rolls over E-selectin were lost with the knockdown of CD34. These data suggest that CD34 plays a key role in tether and sling formation which may be attributed to its role in microvilli on cells since cells where CD34 were knocked down lacked microvilli. Furthermore, using advanced super-resolution microscopy to image single molecular ligand architecture on the cell surface before and after rolling on E-selectin, we found that CD34 forms a nanoscale “ring-like” clustering pattern surrounding other key E-selectin ligands, like CD44, on KG1a cells which is exaggerated following rolling. This “ring-like” localization pattern appears to be a pre-requisite to tether and sling formation from microvilli since disruption of CD34 architecture, results in cells that lack microvilli and the ability to form tethers and slings. Moreover, we observed that tethers and slings show increased concentrations of CD34 on their tethering points implicating a critical role for CD34 at these points which are lost when CD34 is silenced. This work will set the stage to uncover the molecular mechanisms that cells use to migrate in the blood stream and go beyond understanding the role of the canonical selectin ligands individually and consider their role at both the subcellular level relating to their distribution and architecture on cells and their role in transmitting signals.

Keywords: Hematopoietic stem/progenitor cells (HSPC), CD34, Microvilli

TOPIC: KIDNEY

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VASCULOGENESIS IN KIDNEY ORGANOIDS UPON TRANSPLANTATION

van den Berg, Cathelijne W. - *Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Koning, Marije - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Dumas, Sébastien - *Laboratory of Angiogenesis and Vascular Metabolism, VIB Center for Cancer Biology, Leuven, Belgium*
Avramut, Cristina - *Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands*
Koning, Roman - *Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands*
Meta, Elda - *Laboratory of Angiogenesis and Vascular Metabolism, VIB Center for Cancer Biology, Leuven, Netherlands*
Lievers, Ellen - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Wiersma, Loes - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Borri, Mila - *Laboratory of Angiogenesis and Vascular Metabolism, VIB Center for Cancer Biology, Leuven, Belgium*
Liang, Xue - *Lars Bolund Institute of Regenerative Medicine, BGI-Qingdao, Qingdao, China*
Xie, Lin - *MGI, BGI-Shenzhen, China*
Liu, Ping - *MGI, BGI-Shenzhen, China*
Chen, Fang - *MGI, BGI-Shenzhen, China*
Lin, Lin - *Department of Biomedicine, Aarhus University, Aarhus, Denmark*
Luo, Yonglun - *Department of Biomedicine, Aarhus University, Aarhus, Denmark*
Mulder, Jaap - *Department of Pediatrics - Nephrology, Erasmus Medical Center, Rotterdam, Netherlands*
Spijker, Siebe - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Jaffredo, Thierry - *Developmental Biology Laboratory, Sorbonne Université, Paris, France*
van den Berg, Bernard - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*
Carmeliet, Peter - *Laboratory of Angiogenesis and Vascular Metabolism, VIB Center for Cancer Biology, Leuven, Belgium*
Rabelink, Ton - *Department of Internal Medicine - Nephrology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Human induced pluripotent stem cell-derived kidney organoids have potential for disease modelling and to be developed into clinically transplantable auxiliary tissue. However, they lack a functional vasculature and the sparse endogenous endothelial cells (ECs) are lost upon prolonged culture in vitro, limiting maturation and applicability. Here, we use intracoelomic transplantation in chicken embryos to induce and study vasculogenesis in kidney organoids using single-cell RNA sequencing and advanced imaging platforms. We show expansion of human organoid-derived ECs that reorganize into perfused capillaries and form a chimeric vascular network with host-derived blood vessels. Ligand-receptor analysis reveals extensive potential interactions of human ECs with perivascular cells upon transplantation, enabling vessel wall stabilization. Perfused glomeruli display maturation and morphogenesis to capillary loop stage. Our findings demonstrate the beneficial effect of vascularization on not only epithelial cell types, but also the mesenchymal compartment, inducing the expansion of ‘on target’ perivascular stromal

cells which in turn are required for further maturation and stabilization of the neo-vasculature. The vasculogenic capacity of kidney organoids will have to be deployed to achieve meaningful glomerular maturation and kidney morphogenesis in vitro that is required for future clinical application.

Funding Source: Regenerative Medicine Crossing Borders (RegMedXB), Health Holland and The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark'
Keywords: iPSC-derived kidney organoids, Single-cell RNA sequencing, Transplantation

TOPIC: LIVER

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CHROMATIN DYNAMICS AND TRANSCRIPTION FACTOR NETWORKS DRIVING HEPATIC FATE IN IPSC DERIVED HEPATOCYTES

Stephan, Tabea L. - *Terry Fox Laboratory BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada*
Cullum, Rebecca - *Terry Fox Laboratory, BC Cancer Research Institute, Vancouver, BC, Canada*
Drissler, Sibyl - *Terry Fox Laboratory BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada*
Duncan, Stephen - *Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina, Charleston, SC, USA*
Hoodless, Pamela - *Terry Fox Laboratory BC Cancer Research Institute, University of British Columbia, Vancouver, BC, Canada*

Abstract: How the hepatic transcriptome is established during development, including how the underlying chromatin dynamics control cell identity and function is still unclear. The lack of consistency and functionality of iPSC derived hepatocytes suggest a knowledge gap. Here, we describe the changing epigenome during in vitro hepatic differentiation and identify missing links in the transcription factor (TF) network. We hypothesize that genome-wide mapping of enhancers during hepatic differentiation can identify TFs governing a hepatic chromatin landscape in an unbiased approach. By disrupting these TFs using knockdown studies, we will dissect their role in the TF network that controls hepatic identity. We are using in vitro differentiation of iPSCs to hepatic progenitors as a model of liver cell differentiation. By mapping post-translational histone tail modifications across the genome at several time points we developed a comprehensive map of enhancers and promoters and their activation status during hepatic cell differentiation. We found a highly dynamic epigenome with 99% of all enhancers changing their activation status throughout the differentiation. While some enhancers cycle between active and repressed without a clear association with a differentiation stage, others follow a distinct activation pattern. We focus on two groups of enhancers; enhancers primed for activation in endoderm which are not consecutively shutdown as the majority, but activated during further differentiation towards hepatocytes and enhancers which are for the first time activated in hepatoblasts after being quiescent earlier in differentiation. These enhancers appear crucial for hepatic cell fate as they include enhancers regulating the promoters of HNF4a and CEBPa which are master regulators of hepatocyte cell fate amongst others. We are using TF motif enrichment analysis to identify factors directing these enhancer dynamics to then map the binding of identified TFs genome-wide. We further developed an inducible knockdown system to remove TFs of interest during differentiation. A better understanding of the chromatin regulation underlying development will be of value for research into efficient generation of in vitro derived hepatocytes or the regulation of chromatin during liver regeneration and oncogenesis.

Keywords: Enhancer dynamics, Transcription factors, Liver specification

TOPIC: MUSCULOSKELETAL

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CHARACTERIZATION OF THE THREE-DIMENSIONAL GENOME IN HUMAN SKELETAL MUSCLE PROGENITOR CELLS

Romero, Matthew A. - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Woodland Hills, CA, USA*
Nicoletti, Chiara - *Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, USA*
Chien, Peggie - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Saleh, Kholoud - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Gibbs, Devin - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Gane, Lily - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Caputo, Luca - *Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, USA*
Puri, Pier - *Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, USA*
Pyle, April - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*

Abstract: Duchenne Muscular Dystrophy (DMD) is a devastating disease with no cure affecting approximately 1 in 5,000 boys. Stem cell treatments using skeletal muscle stem cells (or satellite cells, SCs) provide great potential for regenerating new muscle and we have developed directed differentiation strategies to generate skeletal muscle cells from human induced pluripotent stem cells (hiPSCs). Our work has shown that hiPSCs generate PAX7+ skeletal muscle progenitor cells (SMPCs) resembling early myogenic cells that align closer to week 7-12 in human development and are not equivalent to adult SCs. We are interested in understanding the key molecular and functional differences that control SMPC versus SC cell states. There is an intense interest in the three-dimensional (3D) organization of the genome and its involvement in cell specific gene regulation. This has led to the discovery of chromatin loops between gene enhancers and promoters as well as self-interacting domains termed topologically associating domains (TADs). Recently published data support a role for the 3D genome in differentiation, however, the differences between the 3D genome in human SMPCs compared to adult SCs is unknown. Hi-C was used to characterize the 3D genome of SMPCs. We found genome-wide 3D configurations were different between hPSCs and SMPCs as was the number of TADs. Interestingly, TAD size was also different between cell types, suggesting dynamic control of TADs during differentiation. When focusing on the PAX7 locus, TAD boundaries differ between cell types further

highlighting the dynamic nature of TADs with respect to cell specific gene expression. Not all muscle specific loci were different between cell types, however, suggesting that some TADs may be pre-established early in the differentiation process while others are established de-novo. With respect to chromatin looping at the PAX7 locus, we found SMPC specific looping between the PAX7 promoter and downstream sequences that were unique in SMPCs. Considering that PAX7 enhancer sequences have yet to be determined, these sequences may serve as candidate enhancers for PAX7. These data, for the first time, characterize the 3D genome of human SMPCs using Hi-C. Moreover, these data provide candidate enhancer sequences for that could provide unique candidates for support of PAX7 SMPCs.

Funding Source: This work is supported by NIH grants R01AR06432706AI (A.D.P.) and R01AR064327-06AI:S1(M.A.R.).
Keywords: Muscle stem cells, Three-dimensional genome, Differentiation

TOPIC: NEURAL

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DEVELOPMENTAL PROGRESSION OF NEURAL PROGENITOR CELLS IN THE HUMAN DENTATE GYRUS

Pastor Alonso, Oier - *Neurology, University of California, San Francisco, CA, USA*
Cheng, Andrew - *Neurology, University of California, San Francisco, CA, USA*
Harris, Jaz - *Neurology, University of California, San Francisco, CA, USA*
Lambing, Hannah - *Neurology, University of California, San Francisco, CA, USA*
Sandoval, Kadellyn - *Neurology, University of California, San Francisco, CA, USA*
Kim, Jaeyeon - *Neurology, University of California, San Francisco, CA, USA*
Bhade, Mohini - *Neurology, University of California, San Francisco, CA, USA*
Chu, Julia - *Neurology, University of California, San Francisco, CA, USA*
Paredes, Mercedes - *Neurology, University of California, San Francisco, CA, USA*

Abstract: Radial glial cells (RGCs) are the primary stem cells driving brain formation. RGCs generate intermediate neural progenitors (INPs) that expand and differentiate into neurons. The mammalian hippocampus (HP), related to learning and memory, harbors RGCs still after birth in a substructure called dentate gyrus (DG). In humans, however, the organization of RGCs during development remains poorly understood and the temporal extent of neurogenesis remains under discussion. We have studied the developing human HP using immunohistochemistry (IHC) and single-nucleus transcriptomics. We performed IHC at gestational week (GW) 14, 18, 22, 30, 39, and 3 weeks after birth labeling astrocytes (S100β) RGCs (Nestin) and INPs (Tbr2). At GW14-GW18, RGCs connected from a limited section of the ventricular region next to the fimbria (potentially equivalent to the dentate neuroepithelium) with the DG, and INPs formed a stream along these RGCs. The fimbria and the subpial zone (between the DG and hippocampal fissure) also showed RGCs that contacted with the DG. Moreover, S100β expression was observed colocalizing with Nestin+ processes coming from the fimbria, suggesting a possible role of S100β as a progenitor marker. The radial structures connecting with the DG were maintained through GW22-GW30 but disappeared at perinatal stages, when Nestin+ fibers were restricted to the granule layer of the DG. Although Nestin expression was observed in the fimbria and adjacent ventricular region perinatally, these fibers were disconnected from the DG. INPs got restricted to the DG even earlier, at GW22, and they gradually decreased their numbers from GW22 onwards. Single nucleus RNA-sequencing of human HP samples at early-mid gestation (GW15, GW18, GW21) early after birth (2 weeks, 4 months, 7 months) and adulthood (38 years) showed the presence of heterogeneous RGC populations in the developing HP. Supporting histological observations, the transcriptomic signature of the HP shifted towards an astrocytic identity over time, at the expense of RGCs. These results highlight the complex organization of RGCs in the early gestational HP and their disappearance over time, leading to a reduced postnatal neurogenic capacity.

Keywords: hippocampus, Radial glia, neurogenesis

TOPIC: PANCREAS

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MULTIOMIC SINGLE CELL ANALYSIS IDENTIFIES MECHANISMS OF HUMAN PANCREATIC ENDOCRINE CELL SPECIFICATION AND BETA CELL MATURATION

Zhu, Han - *Department of Pediatrics, University of California, San Diego, La Jolla, CA, USA*
Wang, Gaowei - *Department of Pediatrics, University of California San Diego, La Jolla, CA, USA*
Nguyen-Ngoc, Kim-Vy - *Department of Pediatrics, University of California San Diego, La Jolla, CA, USA*
Kim, Dongsu - *Department of Pediatrics, University of California San Diego, La Jolla, CA, USA*
Kovsky, Jenna - *Department of Pediatrics, University of California San Diego, La Jolla, CA, USA*
Goss, Georgina - *Centre for Stem Cell and Regenerative Medicine, King's College London, UK*
Miller, Michael - *Center for Epigenomics, University of California San Diego, La Jolla, CA, USA*
Preissl, Sebastian - *Center for Epigenomics, The Regents of the University of California, U.C. San Diego, La Jolla, CA, USA*
Spagnoli, Francesca - *Centre for Stem Cell and Regenerative Medicine, King's College London, UK*
Powers, Alvin - *Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, USA*
Gaulton, Kyle - *Department of Pediatrics, Institute for Genomic Medicine, University of California San Diego, La Jolla, CA, USA*
Sander, Maike - *Department of Pediatrics, Institute for Genomic Medicine and Department of Cellular & Molecular Medicine, University of California San Diego, La Jolla, CA, USA*

Abstract: Human pluripotent stem cell (hPSC)-derived islet cells (SC-islets) hold great promise for diabetes therapy, both as a transplantable cell source and as a model for understanding disease mechanisms. SC-islets consist of endocrine cells that resemble primary islet endocrine cell types, including alpha-, beta-, and delta-like cells. In addition, endocrine cells with intestinal enterochromaffin features (SC-ECs) are produced, which are not found in primary islets. The gene regulatory mechanisms that specify

endocrine cell populations during hPSC differentiation and how closely SC-derived endocrine cells resemble their primary tissue counterparts, however, remains unknown. Here, we combined single-cell genomics and gene regulatory network (GRN) analysis to gain insight into the gene regulatory programs governing SC-islet differentiation. Our analysis identified developmental trajectories and predicted novel transcription factors (TFs) that define the identity of each cell type, as well as the cell-type-specific downstream target genes of TF activity. Furthermore, through integration of single-cell data from SC-islets and primary juvenile and adult human islets, we identified maturation-related transcriptional programs which are less active in SC-beta cells than in primary beta cells. We found that maturation-related transcriptional programs are controlled by signal-dependent TFs, suggesting that physiological signals, including circadian cues, steroid hormones and cytokines, induce beta cell maturation. We validated key predictions from the GRN analysis in the SC-islet differentiation system. Together our findings demonstrate the power of single-cell genomics for informing endocrine cell differentiation strategies from hPSCs.

Funding Source: This project is funded by: NIH U01 DK120429 and UH3 DK122639
Keywords: human PSC-islets, single cell genomics, gene regulatory network

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DIRECTED REPROGRAMMING OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO GLUCOSE-RESPONSIVE INSULIN-PRODUCING CELLS

Jeyagaran, Abiramy - *Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*
Urbanzyk, Max - *Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*
Layland, Shannon - *Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*
Duffy, Garry - *Discipline of Anatomy and the Regenerative Medicine Institute, School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland*
Schenke-Layland, Katja - *Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*

Abstract: Transplantation of stem cell-derived β-cells is a promising therapeutic advancement in the treatment of type 1 diabetes mellitus. A current limitation of this approach is the long differentiation timeline of up to two months that generates a heterogeneous population of pancreatic endocrine cells. To address this limitation, an inducible lentiviral overexpression system of mature β-cell genes was introduced into human induced pluripotent stem cells (hiPSCs). Following selection of the successfully transduced hiPSCs, the cells were treated with doxycycline in pancreatic progenitor induction medium to support their transition towards the pancreatic lineage. qPCR results showed an upregulation of pancreatic β-cell markers Pdx1, Ngn3, Nkx6.1, MafA and MafB after five days of doxycycline treatment, and parallel glucose-stimulated insulin secretion assays (GSIS) demonstrated that the cells were glucose responsive in a monolayer culture. The cells were then aggregated into pseudo-islets for five days where parallel GSIS assays showed an enhanced insulin response to glucose which is comparable to current insulin producing cell protocols. Together, these results suggest a new, simplified, and shortened protocol for the generation of insulin-producing cells from hiPSCs through an inducible lentiviral overexpression system.

Funding Source: The authors acknowledge funding from the European Union's Horizon 2020 framework for the DELIVER project (ID 812865), the Ministry of Science, Research and the Arts of Baden-Württemberg, and the Deutsche Forschungsgemeinschaft.
Keywords: human induced pluripotent stem cells, reprogramming, insulin-producing

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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SEQUENTIAL ACTIVATION OF TRANSCRIPTION FACTORS DRIVES EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION

Kim, Mijeong - *Molecular Biosciences, The University of Texas, Austin, TX, USA*
Kim, Jonghwan - *Molecular Biosciences, The University of Texas at Austin, TX, USA*
Lee, Bum-Kyu - *Biomedical Sciences, University at Albany-State University of New York, Albany, NY, USA*

Abstract: During early human pregnancy, extravillous trophoblasts (EVT) play a central role in placental anchorage and blood vessel remodeling, whose defects are mainly associated with various placental disorders, such as preeclampsia. Despite the essential roles in pregnancy, key factors and the mechanism underlying the EVT differentiation remain largely unknown. Here, we defined two classes of transcription factors (TFs) responsible for EVT differentiation using time-course gene expression profiles combined with enhancer signature. The class 1 factors are active in the early differentiation (early-stage TFs), while the class 2 factors are active in the late-stage of differentiation (late-stage TFs). Depletion of the early- or late-stage TFs resulted in impaired induction of EVT-specific genes and decreased invasion ability, suggesting that they are critical for EVT differentiation and functions. Interestingly, we found that the early-stage TF binds the cis-regulatory elements of the EVT-specific genes that are yet transcriptionally active, implying they may prime the regulatory loci of EVT-specific genes for late-stage activation. Consistent with this, we confirmed that the late-stage TFs replace the bindings of the early-stage TFs in conjunction with the activation of EVT genes in the progression of EVT differentiation, suggesting unexpected collaborations between the early- and late-stage TFs for EVT differentiation. Our findings advance our understanding of the mechanism underlying EVT linage differentiation and provide a clue for diagnostic and therapeutic targets for EVT-differentiation-associated placental complications.

Funding Source: This research was supported by R01HD101512 (NIH) to Dr. Jonghwan Kim.
Keywords: Extravillous trophoblast, Placenta, Super-enhancer

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SIMPLIFIED CULTURE CONDITIONS FOR ESTABLISHING PIG STEM CELL-LIKE CELLS USING SERUM-FREE MEDIA

Choi, Hyerin - *Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea*
Hyun, Sang-Hwan - *Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea*
Zheng, Haomiao - *Veterinary Medical Center and College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Stable pig embryonic stem cells (pESCs) can be an excellent model for understanding human cell fate decisions during early development since pigs are more similar to humans in anatomy and physiology. We developed the simplified culture conditions for establishing pESCs by using a minimum of small molecules in serum-free media. Blastocysts obtained in vitro on day 6 were seeded on the feeder cell layer with DMEM/F10 (1:1), DMEM/F12, and α-MEM medium to compare an efficiency of the pESCs establishment according to the type of the basal medium and small molecules. The small molecule conditions were FGF2 (F), FGF2 + IWR-1 (FI), and FGF2 + IWR-1 + CHIR (FIC). All three basal media showed success attachment of the blastocysts to the feeder layer. On the other hand, colonies formation was observed only in DMEM/F10 and DMEM/F12 conditions. The SOX2 expression in each colony was confirmed using immunostaining. Both DMEM/F10 and DMEM/F12 showed negative results for SOX2 in the F condition, while it was expressed homogeneously in the FI and FIC. pESCs were established under the DMEM/F12 FI, the most defined and simple condition, and examined the pluripotency. When passaging of pESCs as a single cell using TrypLE, they showed high survival. Furthermore, both passages 3 and 7 were positive for alkaline phosphatase activities. The karyotyping result showed a normal karyotype (36+XX). Immunostaining data showed homogenous expression of pluropotency markers OCT4, SOX2, NANOG, and SSEA4. When comparing the expression levels of various genes with serum condition pESCs, the expressions of pluripotency (Oct4, Nanog, and Sox2) and FGF signaling-related genes (bFGF, FGFR1, and FGFR2) were significantly higher. Although further studies are required to examine differentiation potential, these findings demonstrate that FGF2 and IWR-1 are enough to establish pig stem cell-like cells from blastocysts.

Funding Source: This work was supported by grants from the "NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)" and "IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.
Keywords: Pig embryonic stem cells, FGF2, IWR-1

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NONSENSE MEDIATED MRNA DECAY IS REQUIRED FOR TIMELY CELL FATE TRANSITIONS BY FINE-TUNING GENE EXPRESSION AND REGULATING TRANSLATION

Leeb, Martin - *Max Perutz Laboratories Vienna, University of Vienna, Austria*

Abstract: Cell fate transitions depend on balanced rewiring of transcription and translation programmes to mediate ordered developmental progression. Components of the nonsense-mediated mRNA decay (NMD) pathway have been implicated in regulating embryonic stem cell (ESC) differentiation, but the exact mechanism is unclear. Here we show that NMD controls expression levels of the translation initiation factor Eif4a2 and its premature termination codon encoding isoform (Eif4a2PTC). NMD deficiency leads to translation of the truncated eIF4A2PTC protein, which elicits increased mTORC1 activity and translation rates and causes differentiation delays in NMD deficient mouse ESCs. This establishes a previously unknown feedback loop between NMD and translation initiation. Furthermore, our results show a clear hierarchy in severity of target deregulation and differentiation phenotype between NMD-effector KOs (Smg5 KO> Smg6 KO> Smg7 KO), which highlights heterodimer-independent functions for SMG5 and SMG7. Together, our findings expose an intricate link between mRNA homeostasis and mTORC1 activity that must be maintained for normal dynamics of cell state transitions.

Keywords: pluripotency, differentiation, transcription and translation homeostasis

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MULTI-LEVEL EPIGENOME PROFILING REVEALS TEMPORALLY DISTINCT ROLE FOR DNAME AT CELL FATE SPECIFYING ENHANCERS DURING EARLY DIFFERENTIATION

Guerin, Lindsey - *Department of Biochemistry, Vanderbilt University, Nashville, TN, USA*
Hodges, Emily - *Department of Biochemistry, Vanderbilt University, Nashville, TN, USA*

Abstract: The process of cell differentiation involves coordinated regulation of the epigenome on multiple levels, from DNA methylation (DNAme) to chromatin conformation. According to canonical models of enhancer activation, chromatin accessibility (ChrAcc) increases first followed by a decrease in DNAme, ultimately allowing for activation of associated genes. Recent work has established that DNAme removal may not be necessary for transcription, and the dynamic relationship between chromatin regulation and DNAme remains poorly understood. Using ATAC-Me, a method which simultaneously profiles DNAme and ChrAcc, we investigated the regulatory function of DNAme during a densely sampled time course of embryonic stem cell to neural progenitor cell differentiation. We show that ChrAcc responds quickly, and transiently in some contexts, to induction of differentiation with ~38,000 regions displaying dynamic accessibility behavior. Of these, many display concordant changes, where decreases in DNAme accompany increases in ChrAcc. However, ~7,500 regions show discordant epigenetic behaviors. In contrast to ChrAcc, which is modified as early as 6 hours, DNAme dynamics occur primarily during a specific window of time coinciding with increased TET2 expression and peak 5-hydroxymeth-

ylation levels, indicating active removal of DNAme. Our joint profiling methodology combined with dense time course sampling has expanded our knowledge of DNAme's function, highlighting temporally distinct behaviors dominated by DNAme loss. To link these behaviors to regulatory function, we performed transcription factor (TF) footprinting and RNA-seq on the same time course ultimately identifying 214 differentially active TFs. Integration of TF footprints and ATAC-Me data suggest the modification of DNAme levels is not immediately required for alterations in regulatory function of underlying regions. Distinct periods of active demethylation, which generate hypomethylation profiles maintained over time, suggest a role for DNAme as a critical switch in the cell specification process. This work illuminates the kinetics of DNAme modification in relation to dynamic chromatin behaviors, ultimately providing context as to when DNAme exerts its regulatory function.

Keywords: Epigenetics, DNA Methylation, Differentiation

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MINIMAL NUTRITIONAL REQUIREMENTS OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Lyra-Leite, Davi M. - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Copley, Raymond - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Freeman, Phillip - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Shah, Disheet - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Pinheiro, Emily - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Weddle, Carly - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
McKenna, Donald - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Magdy, Tarek - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Fonoudi, Hananeh - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*
Burridge, Paul - *Department of Pharmacology, Center for Pharmacogenomics, Northwestern University, Chicago, IL, USA*

Abstract: Despite more than two decades of work on human pluripotent stem cell culture media, one aspect that has remained largely consistent has been the use of the basal medium DMEM/F12. This medium is one of the most complex common basal media formulae, containing 52 components including 14 essential and 6 non-essential amino acids, eight 'B' vitamins plus myo-inositol and choline, 11 inorganic salts, HEPES, and other more unconventional components such as hypoxanthine, linoleic acid, lipoic acid, putrescine, and thymidine. In addition to its complexity, DMEM/F12 is also unique in its relatively high osmolality. Even though the role of each of DMEM/F12's components is hypothesized, their need for hiPSC growth and maintenance has not been fully demonstrated. Here, building on our prior work that established the optimal concentrations of the B8 supplement, we develop a simplified novel basal media we call BMEM, while also demonstrating that many components of DMEM/F12 are superfluous for hiPSC growth. We further demonstrate that each of these identified 40 components is essential for hiPSC growth and maintenance, while performing titration assessments to identify their optimal concentrations for hiPSC growth. In addition to robust cell proliferation, hiPSC lines cultured in B8 supplemented BMEM have preserved differentiation potential, compared to those kept in B8 supplemented DMEM/F12. This novel basal media is also optimized to eliminate the need for daily hiPSC media changes, allowing for a 'feeding-free' culture methodology, with minimal impact on cell proliferation. BMEM is also suitable for the derivation of hiPSC lines both from control and patient samples, having been validated through the generation of more than 20 hiPSC lines. Lastly, BMEM can easily be produced in-house at a large scale with basic equipment for < 1% of the cost of commercial basal media.

Funding Source: American Heart Association Postdoctoral Fellowship (874276).
Keywords: Cell culture media, Human induced pluripotent stem cells, Nutritional Requirements

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IDENTIFICATION AND FUNCTIONAL STUDY OF 18Q DELETION IN HPSCS

Lei, Yingnan - *Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium*
Krivec, Nuša - *Reproduction and Genetics (REGE), Vrije Universiteit Brussel, Brussels, Belgium*
Couvreu De Deckersberg, Edouard - *Reproduction and Genetics (REGE), Vrije Universiteit Brussel, Brussels, Belgium*
Al Delbany, Diana - *Reproduction and Genetics (REGE), Vrije Universiteit Brussel, Brussels, Belgium*
Sermon, Karen - *Reproduction and Genetics (REGE), Vrije Universiteit Brussel, Brussels, Belgium*
Spits, Claudia - *Reproduction and Genetics (REGE), Vrije Universiteit Brussel, Brussels, Belgium*

Abstract: Human pluripotent stem cells (hPSCs) hold a great promise in regenerative medicine and drug discovery, due to their unlimited capacity for self-renewal and pluripotency. However, hPSCs can exhibit genetic instability and acquire frequently chromosomal abnormalities, that can affect in vitro properties as well as clinical applicability. Deletions of chromosome 18q (18q21.2q.23 and 18q21.32q23) are relatively rare structural chromosomal abnormalities in hPSCs, that have been linked to congenital malformations, intellectual disability, and some types of cancers. Moreover, the impact of 18q deletion on the differentiation capacity of hPSCs is largely unknown. This study aims to examine the functional effects of the 18q deletions during the differentiation of hPSCs into the three embryonic germ layers. Our results from two different isogenic lines of hPSCs showed that cells with 18q deletion (hPSCsdel18q) failed to differentiate properly into the endoderm and ectoderm germ layers, while their differentiation into the mesoderm germ layer was similar to that of normal cells. RNA-sequencing analysis of hPSCsdel18q differentiated into retinal pigmented epithelium (RPE), revealed a potential misspecification of hPSCsdel18q into trophoblast and/or extra-embryonic mesoderm. Furthermore, the losses of 18q mostly span a very large chromosomic region, including SALL3 gene that has been reported previously to regulate the differentiation propensity of hiPSCs. We hypothesized that SALL3 could be a driving gene that modulates the differentiation capacity of hPSCsdel18q. To further investigate our hypothesis, we will perform SALL3 loss and gain of function studies in several lines of hPSCs and verify if SALL3 is the potential link between the 18q deletions and the impaired differentiation of hPSCs. These findings will expand our knowledge on the functional consequences of genetic

aberrations in hPSCs-derived cells and the safety of their usage in clinical translation medicine.

Keywords: human pluripotent stem cells, chromosome 18q, differentiation capacity

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GENE EXPRESSION SIGNATURES OF EXTRACELLULAR MATRIX AND INTEGRINS DURING HUMAN OTIC SENSORY DIFFERENTIATION FROM PLURIPOTENT STEM CELLS

Zine, Azel - *Laboratory of Bioengineering and Nanoscience, University of Montpellier, France*
Messat, Yassine - *Laboratory of Bioengineering and Nanoscience, University of Montpellier, France*
Lahlou, Hanae - *Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA*
Steinacher, Claudia - *Inner Ear Laboratory, Department of Otorhinolaryngology, Medical University Innsbruck, Austria*
Schrott-Fischer, Anneliese - *Medical University Innsbruck, Inner Ear Laboratory, Department of Otorhinolaryngolog, Innsbruck, Austria*

Abstract: Two approaches have been subjected to restore inner ear HCs that do not regenerate, i.e., gene and stem cell-based cell therapies. The stem cell approach requires the robust production and characterization of otic sensory progenitor cells. To gain new insights into early human otic neurosensory lineage, we analyzed transcriptomic data from otic sensory cells differentiated from human induced pluripotent stem cells (hiPSCs) by a previously described method. We identified genes and biological networks not previously described to occur in the human otic sensory cell lineage. These analyses identified and ranked genes known to be part of the otic sensory lineage program (SIX1, EYA1, GATA3), in addition to a number of novel genes encoding extracellular matrix (ECM) (COL3A1, COL5A2, FN) and integrin (ITG) receptors (ITGAV, ITGA4, ITGA) for ECM molecules. The results were confirmed by quantitative PCR analysis of a comprehensive panel of genes differentially expressed during the time course of hiPSC differentiation in vitro. Results were validated by immunochemistry for select otic and ECM/ITG gene markers in the human fetal inner ear. Our screen shows ECM and ITG gene expression changes coincident with hiPSC differentiation towards human otic neurosensory cells. In summary, we report a critical role of ECM-ITG interactions with otic neurosensory lineage genes in early neurosensory development and cell fate determination in the human fetal inner ear.

Keywords: induced pluripotent stem cells, extracellular matrix/integrins, human fetal inner ear

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ESTABLISHMENT OF SELF-RENEWING AND MULTIPOTENT TROPHOBLAST STEM-LIKE CELLS FROM HUMAN PRIMED PLURIPOTENT STEM CELLS

Jang, Yu Jin - *Molecular Biosciences, University of Texas, Austin, TX, USA*
Kim, Mijeong - *Molecular Biosciences, The University of Texas at Austin, TX, USA*
Lee, Bum-Kyu - *Biomedical Sciences, University at Albany-State University of New York, Albany, NY, USA*
Kim, Jonghwan - *Molecular Biosciences, The University of Texas at Austin, TX, USA*

Abstract: A detailed understanding of human placental development is important, as improperly developed placenta may lead to fetal-maternal disease. Despite its importance, studies of early placentation have been constrained by a limitation of early placenta resources and a lack of appropriate in vitro model systems. Human trophoblast stem cells (TSCs) are considered a reliable model system but also come with ethical issues as they are derived from developing embryos or early-stage placentas. Although there have been many successful efforts to generate human trophoblast stem-like cells (TSLCs) from naïve pluripotent stem cells (PSCs), the conversion of TSLCs from primed PSCs is still debated. Here, we generate human TSLCs from primed PSCs by culturing in trophoblast stem cell culture medium (TSCM) with a short-term treatment of bone morphogenetic protein 4 (BMP4). These TSLCs are comparable to human TSCs derived from early placenta in terms of morphology, global gene expression profile, and self-renewal capacity. We show that the cells have multipotency which can differentiate into functional syncytiotrophoblasts (ST) and extravillous trophoblasts (EVT). This simple and powerful procedure of generating TSLCs provides a tremendous opportunity to understand human placenta development and pathogenesis of placenta-related diseases.

Funding Source: This work was supported by R01HD101512 (NIH/NICHD) and Preterm Birth Research Grant (1017294, Burroughs Wellcome Fund) to J.K.
Keywords: Human primed pluripotent stem cells, Human trophoblast stem cells, Bone morphogenetic protein 4

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ELEVATED TRACTIONS AROUND EPISC COLONIES TRIGGER HETEROGENEOUS DIFFERENTIATION

Chowdhury, Farhan - *Mechanical, Aerospace, and Materials Engineering, Southern Illinois University Carbondale, IL, USA*
Amar, Kshitij - *Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
Saha, Sanjoy - *Mechanical Engineering, University of Notre Dame, North Bend, IN, USA*
Debnath, Avishek - *Mechanical Engineering and Materials Science, Washington University in St. Louis, MO, USA*
Weng, Chun-Hung - *CREOL, College of Optics and Photonics, University of Central Florida, Orlando, FL, USA*
Han, Kyu Young - *CREOL, College of Optics and Photonics, University of Central Florida, Orlando, FL, USA*

Abstract: In addition to soluble factors, it is now well established that pluripotent stem cell fate (self-renewal and differentiation) is regulated by mechanical forces. These mechanical signals are transduced into cells via cell-extracellular matrix interactions. However, upon differentiation, not all pluripotent mouse epiblast stem cells (EpiSCs) within the same colony respond in the same manner resulting in heterogeneous cell differentiation. Since EpiSCs are cultured in clusters and the mechanical induction of differentiation results in even severe heterogeneous cell populations. Many studies correlate this heterogeneity to the variation of active signaling pathways. Nevertheless, the biophysical aspects of differentiation are not thoroughly considered. Here, we show that the heterogeneity of EpiSCs arises due to differences in the physical size of the colony and varying levels of interactions between the cell and the extracellular matrix. With confocal

imaging, we show that cells in the colony center remain elevated by 1-2 µm from the surface. Traction force measurements of the cells within the EpiSC colonies show that peripheral cells indeed generate large traction while the colony center cells do not. Interestingly, ~50% of the colonies studied show that the peripheral cells generate an unusual rotational moment that separates the colony center which was also validated by a finite element analysis model. Together, our results show that the larger the colony size induces higher traction including unusual rotational moments causing the lift-off in the colony center and thus creating a large heterogeneity during cell differentiation.

Funding Source: NIH grants GM 148440 (F.C.) and GM 131163 (K.Y.H.)
Keywords: Epiblast stem cells, biophysical mechanism, heterogeneous differentiation

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DISTAL REGULATION OF THE PLURIPOTENCY 3D GENOME IN GENETICALLY DIVERSE MOUSE EMBRYONIC STEM CELLS IMPACTS DIFFERENTIATION PROPENSITY

Baker, Christopher L. - *Research, The Jackson Laboratory, Bar Harbor, ME, USA*
Byers, Candice - *Research, The Jackson Laboratory, Bar Harbor, ME, USA*
Fortin, Haley - *Genetics, Graduate School of Biomedical Sciences, Tufts University, Bar Harbor, ME, USA*
Spruce, Catrina - *Research, The Jackson Laboratory, Bar Harbor, ME, USA*

Abstract: Genetically diverse pluripotent stem cells (PSCs) display varied, heritable, responses to differentiation cues, a significant hurdle in applying PSCs for personalized medicine. By harnessing these disparities through derivation of embryonic stem cells from a mouse genetic reference panel, along with C57BL/6J (B6) and DBA/2J (D2) parental strains, we previously demonstrated genetically determined biases in lineage commitment and identify major regulators of the pluripotency epigenome. Upon transition from naïve to formative pluripotency, B6 quickly dissolved naïve networks and adopted gene expression modules indicative of neuroectoderm lineages; whereas D2 retained aspects of naïve pluripotency with little bias in differentiation. Genetic mapping identified 6 major trans-acting quantitative trait loci (QTL) that co-regulated chromatin accessibility and gene expression, indicating an epigenomic regulatory system that impacts cell state transition. We validated these genetically determined differentiation propensities through spontaneous formation of embryoid bodies coupled with single cell transcriptomics. As predicted, B6 showed differentiation biases towards neuroectoderm and D2 towards definitive endoderm, among other cell fate differences. To understand the molecular mechanisms by which these trans-QTL impact chromatin organization we employed HiChIP to measure differential 3D genome contacts at regulatory elements. We found that the allelic identity of the QTL determined differential 3D contacts at distal chromatin targets throughout the genome. Epigenetic differences between B6 and D2 identified a negative correlation with the repressive mark H3K9me3 and 3D contacts. In support of genetically determined heterochromatin formation, these loci also showed an enrichment for TRIM28 binding, a scaffold protein responsible for H3K9me3 deposition and formation of heterochromatin. Together these data, along with the identity of genes underlying the QTL, implicate a class of highly diverse DNA binding proteins known as KRAB-ZFPs as the causal factors regulating 3D genome organization, gene expression, and potentially cell fate differences between genetically diverse PSCs.

Funding Source: This work was funded through The National Institute of General Medical Sciences (NIGMS) grant R35GM133724.
Keywords: Genetic variation, mESC, 3D chromatin

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DECOUPLING OF TRANSCRIPTIONAL ACTIVATION FROM ENHANCER CHROMATIN DYNAMICS DURING THE NAÏVE TO FORMATIVE PLURIPOTENT TRANSITION

Boileau, Ryan - *Developmental and Stem Cell Biology, University of California, San Francisco, CA, USA*
Chen, Kevin - *Developmental and Stem Cell Biology, University of California, San Francisco, CA, USA*
Blelloch, Robert - *Department of Urology, University of California, San Francisco, CA, USA*

Abstract: Cell type specific gene expression throughout development is controlled by cis regulatory enhancers and chromatin regulators such as histone modifiers. During early embryonic stem cell (ESC) differentiation, the enhancer landscape is dramatically rewired. How this rewiring occurs is not well understood. Here, we aimed to understand the role of the MLL3 and MLL4 H3K4 monomethyltransferases in this process. H3K4 monomethylation (H3K4m1) is thought to be one of the early events in a stepwise process of enhancer activation followed by H3K27 acetylation (H3K27ac) and eventually transcriptional activation of the enhancer target gene. To test this model, we characterized the impact of combined MLL3/4 loss on enhancer and gene activation during ESC differentiation from the naïve to formative pluripotent states. MLL3/4 loss had little impact on H3K4m1 at sites that retained this mark, yet was essential for nearly all sites that either lost or gained H3K4m1 during the transition. Furthermore, while a gain of monomethylation was required for subsequent H3K27ac at a subset of enhancers, many enhancers gained H3K27ac independent of MLL3/4 activity. The transcriptional up-regulation of the vast majority of genes neighboring the impacted enhancers was unaffected by MLL3/4 and the associated loss of the monomethylation mark. Instead, there was a greater impact of MLL3/4 loss on the decrease in expression of genes near enhancers that normally lose H3K4 monomethylation during differentiation. These results challenge the canonical model that H3K4me1 precedes H3K27ac in the activation of enhancers and target genes thus refining our understanding of the factors necessary to rewire the epigenome during cell fate transitions.

Keywords: Chromatin, Epigenetics, Pluripotent Transition

TOPIC: KIDNEY

147

DIRECT CELLULAR REPROGRAMMING OF MAFB-EGFP FIBROBLASTS INTO PODOCYTES

Kim, Doh Kyung - *Molecular and Computational Biology, University of Southern California, Los Angeles, CA, USA*
McMahon, Andrew - *Development, Stem Cells and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Tran, Tracy - *UCLA Broad Stem Cell, UCLA, Los Angeles, CA, USA*

Abstract: Chronic kidney disease results in end-stage renal failure, in which the essential role of blood filtration is compromised. The key cell responsible for filtration is podocyte. Podocytes possess foot processes that form a selective filtration barrier essential for the movement of large molecules into the renal filtrate. Despite the heavy implication of podocytes in kidney function, there is no in vitro model that recapitulates in vivo podocytes; current in vitro primary cultures or cell lines rapidly lose a normal podocyte transcriptional profile and cellular features such as foot processes and their associated structural proteins, and an additional problem of donor availability arises for obtaining primary podocytes. Given the need for an accurate in vitro model of podocytes, we aim to generate podocytes that contain structural and functional characteristics of in vivo counterparts by direct reprogramming of human fibroblasts. Through snATAC-seq and scRNA-seq on human fetal and adult renal corpuscule, the McMahon lab identified 11 transcription factors (TFs) important for podocyte development and/or maintenance. In vivo knock out of these candidates results in dysfunctional podocytes, substantiating their role in podocyte specification and/or function. We hypothesized that the ectopic expression of the 11 TFs will directly reprogram human fibroblasts into podocytes. Here, we report that the ectopic expression of the 11 TFs by lentiviral infection into fibroblasts induces transcriptome and morphological changes. The starting fibroblasts have a MAFB-eGFP transgene, in which eGFP acts as a visual marker for MAFB, a podocyte specific TF exclusively expressed in podocytes during development or in mature glomeruli. Infection of fibroblasts with TF encoding lentiviruses results in ~5% eGFP+ cells on D5 post infection, indicative of MAFB expression, and upregulation of other podocyte markers. In addition, eGFP+ cells exhibit elongated nuclei and morphology, distinguishable from those of the starting cell population. Currently, we are in the process of characterizing and acquiring more mature versions of the reprogrammed cells. Our ultimate goal is to assess functional and structural quality of the reprogrammed cells and to optimize the protocol by identifying the minimal set of TFs to generate podocytes.

Keywords: Direct Reprogramming, Podocytes, Transcription Factors

TOPIC: EARLY EMBRYO

901

MESENDODERM PROGENITOR CELLS IN MAMMALIAN EMBRYO, WHERE AND WHEN DO THEY EXIST?

Masamsetti, Pragathi V. - *Embryology Research Unit, Children's Medical Research Institute, Westmead, Australia*
Kim, Hani Jieun - *Computational Systems Biology, Children's Medical Research Institute, Westmead, Australia*
Sun, Jane - *Embryology Unit, Children's Medical Research Institute, Westmead, Australia*
Aryamanesh, Nader - *Embryology Unit, Children's Medical Research Institute, Westmead, Australia*
Osteil, Pierre - *Embryology Unit, Children's Medical Research Institute, Westmead, Australia*
Yang, Pengyi - *Computational Systems Biology, Children's Medical Research Institute, Westmead, Australia*
Tam, Patrick - *Embryology Unit, Children's Medical Research Institute, Westmead, Australia*

Abstract: Multipotent embryonic cells are capable of contributing to a multitude of cell types in the body. As the development progresses, these progenitor cells become restricted in lineage potential for a specific range of tissue derivatives. At gastrulation, the epiblast cells are allocated to the progenitor cells for the ectoderm, the mesoderm, and the endoderm, which is driven by lineage-specific transcriptional activity and regionalized signaling input. However, when and whereabouts of the allocation of the progenitors of specific germ layers and the trajectory of their differentiation towards multiple lineages are not clearly understood. Previous studies conducted in nematode and zebrafish embryos identified a subset of germ layer progenitors that display dual propensity for mesoderm and endoderm. The existence and lineage propensity of such bipotential mesendoderm progenitors in mammalian embryos remains unclear. Our study focuses on the identification of the putative bipotential progenitor cells of mesoderm and endoderm in mouse embryos using single-cell transcriptome analysis and imaging of the lineage descendants of these progenitors in embryos and in vitro embryo models. Identification and characterization of the bipotential progenitors will enable the efficient generation of biologically relevant cell types for tissue engineering applications.

Keywords: Mouse early embryo development, Lineage tracing, Mesendoderm Progenitor cells

TOPIC: EPITHELIAL_GUT

903

DEVELOPMENT OF AN INSIDE-OUT HUMAN INTESTINAL ORGANOID MODEL

Kakni, Panagiota - *MERLN Institute, Maastricht University, Maastricht, Netherlands*
López-Iglesias, Carmen - *The Maastricht Multimodal Molecular Imaging Institute, M4i, Maastricht University, Maastricht, Netherlands*
Habibović, Pamela - *MERLN Institute, Maastricht University, Maastricht, Netherlands*
Truckenmüller, Roman - *MERLN Institute, Maastricht University, Maastricht, Netherlands*
Giselbrecht, Stefan - *MERLN Institute, Maastricht University, Maastricht, Netherlands*

Abstract: For over a decade, intestinal organoids have constituted a powerful tool for studying intestinal physiology and diseases, since they recapitulate the in vivo architecture, function and cell composition with great fidelity. However, studies related to interactions of the epithelium with luminal contents (e.g. apical transporters, microbes, nutrient uptake etc.) are limited because of the enclosed position of the apical surface in the organoid lumen. Access to it, usually requires labour-intensive methods like microinjections or disruption of the 3D architecture. In this study, we developed for the first time, a method to reverse the polarity of human pluripotent stem cell (PSC)-derived organoids. By culturing organoids in a suspension system, the apical surface of the organoids is facing outwards to the surrounding culture media and the basal surface facing inwards towards the lumen. We demonstrate that these novel PSC-derived inside-out organoids maintain proper apico-basal polarity using fluorescence and electron microscopy. Diffusion assays verify that the barrier integrity remains intact, thus the epithelial functionality is assured. Polarized nutrient absorption is validated with a fatty acid uptake assay, where only apical transport proteins absorb the fatty acid analog C1-BODIPY-C12. Finally, similar to basal-out, apical-out in-

testinal organoids differentiate into the major intestinal cell types. Reversing the polarity of intestinal organoids qualifies them for a broad range of applications including, but not limited to the study of complex host-microbiome/ pathogen interactions, nutrient uptake and drug metabolism. Especially in the case of PSC-derived organoids, early stages of microbial colonization and pathogen infections can be studied as well, which is particularly important since the gut microbiota development remains poorly understood.

Keywords: intestinal organoids, apicobasal polarity, gastrointestinal model

TOPIC: HEMATOPOIETIC SYSTEM

905

MITOCHONDRIAL HETEROGENEITY IN ENDOTHELIAL TO HEMATOPOIETIC TRANSITION (EHT) DURING MOUSE EMBRYONIC HEMATOPOIESIS

Prakash, Aishwarya - *Molecular Biology and Genetics Unit, JNCASR, Bengaluru, India*
Inamdar, Maneesha - *Molecular Biology and Genetics Unit, JNCASR, Bengaluru, India*

Abstract: Immunophenotypically defined hematopoietic stem cells (HSCs) exhibit molecular and functional heterogeneity. Mitochondrial heterogeneity impacts fate and lineage choice of the adult HSCs, however its role during Endothelial to Hematopoietic Transition (EHT) remains unexplored. The evolutionary conserved mitochondrial proteins Asrij/OCIAD1 and OCIAD2 actively regulate mitochondrial activity and dynamics. Asrij levels vary within the HSC pool, while OCIAD2 has a relatively uniform expression. Notably, Asrij is essential for maintaining mouse HSC quiescence, while OCIAD2 has a role in erythropoiesis. However, the contribution of OCIAD proteins towards the origin of hematopoietic/HSC heterogeneity has not been investigated. Hence, we undertook a spatiotemporal mapping of OCIAD proteins in the Aorta Gonad Mesonephros (AGM) and in yolk sac hematopoiesis to elucidate their potential role in determining HSC fate. We observed that OCIAD protein expression is not uniform and overlaps only partially in embryonic HSCs. We also observed that OCIAD proteins are expressed in the hemogenic endothelium and are further enriched in HSC clusters during early emergence in the embryo. Concomitantly, emerging HSCs show increased mitochondrial content compared to hemogenic endothelium, suggesting a correlation with promoting EHT. Our study investigates the role of mitochondrial distribution and morphology regulators in determining early HSC fate decisions.

Keywords: HSC heterogeneity OCIAD proteins, Mitochondrial regulation of hematopoiesis, Endothelial to Hematopoietic Transition

TOPIC: NEURAL

907

IN VITRO NEURONAL MATURATION AND SYNAPTOGENESIS IN TRANSCRIPTION FACTOR-INDUCED HUMAN IPSC-DERIVED NEURONS

Lin, Waka - *Biomedical Research and Development, Ricoh Company, Ltd., Kawasaki-shi, Japan*
Watanabe, Hikaru - *Biomedical Research and Development Department, Ricoh Company, Ltd., Kawasaki-shi, Japan*
Yamada, Saki - *Biomedical Research and Development Department, Ricoh Company, Ltd., Kawasaki-shi, Japan*
Shiomoto, Shusaku - *Biomedical Research and Development Department, Ricoh Company, Ltd., Kawasaki-shi, Japan*
Sekino, Yuko - *Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan*

Abstract: The development of efficient and robust methods to produce induced pluripotent stem cells (iPSC)-derived neurons holds great promise for advancing disease modeling and regenerative medicine. The induction of neuronal differentiation by the expression of neurogenic transcription factors (TF) is becoming increasingly popular since it allows the generation of human neurons in a much shorter time than traditional culture methods. On the other hand, synapse maturity has often been reported to be incomplete or to require substantially longer culture times. In this study, we characterize the ability of TF-induced neurons to undergo the transcriptional and morphological changes associated with the late-stage development of excitatory synapses. To examine the maturation process in TF-induced iPSC-derived neurons, we performed a time-course evaluation of neuronal marker expression and electrophysiological properties for 3 months after induction of differentiation. Transcriptome analysis by RNA sequencing (RNA-seq) confirmed the upregulation of neurogenic and synaptic genes in long-term cultures. Immunocytochemistry also showed a marked increase of dendrite elongation and formation of presynaptic vesicles after 2 to 3 months of culture. We further evaluated the subcellular localization of drebrin, an actin-binding protein involved in the morphology and dynamics of dendritic spines. Drebrin distribution was shown to change over time, similarly to molecular events known in rodent hippocampal neurons, with a transition to a mature brain-specific isoform that accumulates into postsynaptic clusters. Finally, synaptic functionality was evaluated by visualizing the effects of glutamate stimulation on drebrin cluster density and measuring neuronal network activity on microelectrode arrays (MEA). Our results showed for the first time that TF-induced iPSC-derived neurons can reach dendritic spine maturation, which is essential for postsynaptic efficacy and plasticity. Optimizing culture conditions for synapse maturation would prove useful for the future development of in vitro models that are physiologically relevant to higher brain functions and cognitive disorders.

Keywords: iPS cell, synapse, RNA-Seq

909

EARLY HUMAN FETAL NEURAL PRECURSORS CELLS REVEAL HIGH SELF-RENEWAL CAPABILITY AND BROAD NEURAL DIFFERENTIATION POTENTIAL

Günther, Katharina - *Institute of Molecular Biology, University of Innsbruck, Austria*
Thier, Marc - *HI-Stem, German Cancer Research Center, Heidelberg, Germany*
Tisch, Marcel - *Institute of Molecular Biology, University of Innsbruck, Austria*
Wörsdörfer, Philipp - *Institute of Anatomy and Cell Biology, University of Würzburg, Germany*
Suarez Cubero, Marta - *Institute of Molecular Biology, University of Innsbruck, Austria*
Wischmeyer, Eberhard - *Medical Faculty OWL, University of Bielefeld, Germany*
Schlaiß, Tanja - *Department of Gynecology and Obstetrics, University Hospital Würzburg, Germany*
Liebscher, Simone - *Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*
Schenke-Layland, Katja - *Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, Germany*
Wagner, Nicole - *Institute of Anatomy and Cell Biology, University of Würzburg, Germany*
Ergün, Süleyman - *Institute of Anatomy and Cell Biology, University of Würzburg, Germany*
Trumpp, Andreas - *HI-Stem, German Cancer Research Center, Heidelberg, Germany*
Edenhofer, Frank - *Institute of Molecular Biology, University of Innsbruck, Austria*

Abstract: Recently, we reported direct reprogramming of somatic cells into neural plate border stem cells (NPBSC) differentiating in CNS and neural crest progenies. Although NPBSCs resemble cells of anterior hindbrain region in neurulation-stage embryos, it was unclear to which extent a physiological correlate exists. Here, we aimed to stabilize early neurulation-stage stem cells from primary tissue. Thus, we isolated human fetal brain tissue (7 -11 wpc) and employed a defined medium modulating signaling pathways (SHH, WNT, FGF) orchestrating neurodevelopment. We identified conditions stabilizing highly proliferative, homogenous fetal neural precursor cells (fNPCs). fNPCs exhibit a non-polarized morphology and an early neuroepithelial profile including expression of SOX1, PAX6, Nestin, SOX2 and ZO-1 judged by immunofluorescence and qRT-PCR. Flow cytometry revealed CD133, CXCR4 and PSA-NCAM-expressing cells. Notably, fNPCs can be monoclonally expanded (>45 passages) maintaining a primitive NPC phenotype and a normal karyotype. For further characterization, we performed a transcriptomic profiling of poly/mono-clonal fNPCs using scRNA seq. As expected, we observed little variance between samples. GO enrichment revealed genes related to neurodevelopment and neural tube formation. fNPCs expressed not only neuroepithelial (TJP1, HES1), but also radial glia (VIM, CDH) and NPB genes (SOX3, ZIC1). Further data suggest patterning towards ventral regional identity marked by upregulated NKX6-1 and SFRP2 and downregulated NKX2.2. The differentiation analysis of fNPCs unraveled strong neurogenic potential. Presence of astrocytes and oligodendrocytes after targeted differentiation and transplantation confirms trilineage potential. Electrophysiology revealed spontaneous action potentials and immunofluorescence indicated GABAergic, glutamatergic and dopaminergic subtypes. Further, synapse formation was detected by immunostainings and ultrastructural examination. fNPCs differentiate to sensory neurons as shown for NPBSCs. Together, our data suggest thus far unknown primitive fNPCs with broad CNS and neural crest differentiation capacity. Being instrumental to elucidate mechanisms of early neurodevelopment, they represent a novel source for cell replacement and drug screening studies.

Keywords: neural stem cells, fetal neural precursor cells, single cell RNA sequencing

TOPIC: PLURIPOTENT STEM CELLS

911

NOVEL STIFFNESS-TUNABLE HYDROGEL-SANDWICH CULTURE METHOD CONTROLS THE MECHANORESPONSE OF INDUCED PLURIPOTENT STEM CELL-EMBRYOID BODIES

Nattasit, Praphawi - *Molecular and Regenerative Prosthodontics, Graduate School of Dentistry, Tohoku University, Sendai, Japan*
Niibe, Kunimichi - *Molecular and Regenerative Prosthodontics, Graduate School of Dentistry, Tohoku University, Sendai, Japan*
Yamamoto, Masaya - *Department of Materials Processing, Graduate School of Engineering, Tohoku University, Sendai, Japan*
Egusa, Hiroshi - *Molecular and Regenerative Prosthodontics, Graduate School of Dentistry, Tohoku University, Sendai, Japan*

Abstract: Mechanical force during the developing stage of embryonic stem cells influences robust cell migration and tissue-folding during embryogenesis thereby regulating embryonic stem cell fate and patterning. Both signaling ligands and mechanical stimuli reportedly control the differentiation of induced pluripotent stem cells (iPSCs) into specific lineages. The role of mechanical stress and 2D-substrates mimicking local tissue stiffness in regulating stemness and differentiation of iPSCs cultured in a 2D-monolayer has recently been revealed. However, the effect of microenvironment stiffness in 3D-iPSC aggregates or embryoid bodies (EBs) remains to be elucidated. This study investigated the effect of mechanical cues on cellular response in mouse iPSC-derived EBs (miPSC-EBs) using a stiffness-tunable hydrogel-sandwich culture (HSC). miPSC-EBs were cultured under three different HSC conditions, including hard-HSC (54.3 kPa), moderate-HSC (28.1 kPa), and soft-HSC (5.1 kPa), with a standardized gel weight, and a free-floating culture was used as control. Cell proliferation was reduced in miPSC-EBs cultured for two days under hard- and moderate-HSC compared to that under soft-HSC and floating culture conditions. However, miPSC-EBs cell viability in all HSC groups was comparable to that in the floating culture. Nuclear translocation of YAP, a mechanosensitive transcriptional factor of the Hippo signaling pathway, was observed in all HSC groups. In contrast, YAP was found localized in the cell cytoplasm in the floating control culture and the active form of YAP was upregulated in a stiffness-dependent manner. In addition, cells under hard- and moderate-HSC conditions exhibited a dense and interconnecting F-actin organization, whereas the soft-HSC and floating groups exhibited a sparse and unorganized F-actin network. The present study demonstrates that HSC not only affects cell proliferation but also alters cytoskeleton arrangement and activates a mechanosensitive pathway in miPSC-EBs. The established novel HSC allows spatiotemporal control of the microenvironment surrounding the 3D-cell aggregates including EBs and organoids. The HSC method also provides a feasible platform for investigating

the role of mechanical cues on pluripotency and differentiation of pluripotent stem cells.

Funding Source: Grant-in-Aids for Scientific Research (C: 19K10220, K.N. and H.E.; B 19H03840, H.E. and K.N.), Challenging Exploratory Research (18K19630, K.N. and H.E.) from the Japan Society for the Promotion of Science.
Keywords: Hydrogels, Induced pluripotent stem cells, Mechanoresponse

913

ELUCIDATING THE ROLE OF THE CELL CYCLE IN HUMAN EMBRYONIC STEM CELL DIFFERENTIATION USING MULTIPLEXED IMAGING

Shahir, Jamshaid - *Genetics, University of North Carolina at Chapel Hill, Carrboro, NC, USA*
Zahabioun, Amirsaman - *Biology, University of North Carolina at Chapel Hill, NC, USA*
Beltran, Adriana - *Genetics, University of North Carolina at Chapel Hill, NC, USA*
Wolff, Samuel - *Genetics, University of North Carolina at Chapel Hill, NC, USA*
Purvis, Jeremy - *Genetics, University of North Carolina at Chapel Hill, NC, USA*

Abstract: Human embryonic stem cells (hESC) display distinct cell cycle properties from those of somatic cells. The extent to which pluripotency sustains these unique cell cycle behaviors - and vice versa - is not well understood. Understanding how hESCs balance self-renewal and differentiation is key to improving stem cell therapeutics, particularly in the area of directed differentiation where the goal is to generate a high yield of specific cell types. Although single-cell RNA sequencing has enabled detailed studies of differentiation and early embryonic development, this approach is not ideal for studying cell cycle events that are primarily governed by protein turnover and post-translational modifications. Here, we employed a multiplexed imaging approach called iterative indirect immunofluorescence imaging, or 4i, to measure a panel of cell cycle, pluripotency, and germ layers proteins at single-cell resolution in BMP4-treated hESCs. High-content image analysis produced a high-dimensional proteomic signature representative of each cell's unique "identity" and cell cycle state. These data were projected into a lower-dimensional state for visualization and interpretation. Our preliminary results capture dynamics of the cell cycle in hESCs, and reveal how proliferation and arrest programs are remodeled as hESCs undergo differentiation, providing a framework to study the role of the cell cycle in stem cell differentiation.

Keywords: cell cycle, multiplexed imaging, embryonic stem cells

POSTER SESSION III: ODD

3:00 PM – 4:00 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: CARDIAC

203

DEVELOPMENT OF NOVEL SOLID LIPID NANOPARTICLES TO IMPROVE GENOME EDITING IN CARDIAC MUSCLE

Neiman, Gabriel - *California Institute for Quantitative Biosciences, University of California, Berkeley, CA, USA*
Han, Hesong - *Department of Bioengineering and Innovative Genomic Institute, UC Berkeley, CA, USA*
Siemons, Brian - *California Institute for Quantitative Biosciences, UC Berkeley, CA, USA*
Murthy, Niren - *Department of Bioengineering and Innovative Genomic Institute, UC Berkeley, CA, USA*
Healy, Kevin - *Departments of Bioengineering, and Materials Science and Engineering, UC Berkeley, CA, USA*

Abstract: Gene therapy is a promising modality for the treatment of inherited and acquired cardiovascular diseases. In vivo delivery of the CRISPR/Cas9 tool can be exploited for the correction of cardiac mutations such as long QT syndrome. However, genome editing in the heart suffers from low transfection efficiency of non-proliferative cardiomyocytes and delivery through extracellular barriers of 3D cardiac muscle. Non-viral vectors like solid lipid nanoparticles (LNPs) hold great promise in overcoming these limitations and can make a significant breakthrough in genetic medicine. This work aimed to identify a solid LNP formulation that can diffuse within dense cardiac muscle, transfect the mRNA of interest, and edit the genome in a more efficient manner than current strategies having the goal of repairing cardiac genetic diseases. First, different LNP formulations containing acid degradable PEG-lipids were tested, resulting in 80% of GFP mRNA transfection efficiency in a monolayer of human iPSC-derived cardiomyocytes (hiPSC-CM). Then, to assess the gene editing efficiency, CRE mRNA was delivered in the LNPs to remove a STOP cassette flanked by LoxP sequences which is preventing eGFP expression. Gene editing efficiency was 60% in 2D hiPSC-CM. A specific formulation of solid LNPs containing 2kDa PEG-lipid 5% was able to successfully deliver Cas9 mRNA/sgRNA in hiPSC-CM and nearly 20% of them were knockout for a control gene. Preliminary data also showed that the STOP cassette was excised in a small hiPSC-CM population after Cas9 mRNA/multiple sgRNA delivery. In parallel, a cardiac microphysiological system (cardiac MPS) was used as an in vitro platform for investigating the gene editing efficacy of different LNPs formulations in 3D cardiac muscle derived from hiPSC-CM. A specific LNP formulation with 2kDa PEG-lipid was highly diffusive in the 3D muscle in the cardiac MPS. Ongoing work focuses on optimization of the delivery system to perform gene editing in the 3D cardiac muscle. Our work demonstrates that LNPs can diffuse within dense 3D cardiac muscle, can transfect cardiac cells with high efficiency, and has the potential to permanently modify disease-causing genes in patients.

Keywords: hiPSC-derived cardiomyocytes, cardiac muscle, gene editing

TOPIC: EPITHELIAL_SKIN

205

PRECISE AND EFFICIENT EDITING OF THE COL7A1 GENE IN RDEB DERIVED IPSCS WITH CRISPR/CAS9 AND PRIME EDITING

Inen, Jeffrey - *University of Colorado Bioengineering, The Gates Center for Regenerative Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Han, Chann - *SCB&DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Bilousova, Ganna - *SCB&DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
McGrath, Patrick - *Section of Developmental Biology, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Kogut, Igor - *SCB&DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*
Bilousova, Anya - *SCB&DM Core, The Gates Center for Regenerative Medicine, Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

Abstract: Recessive Dystrophic Epidermolysis Bullosa (RDEB) is an incurable genetic skin disease that subjects afflicted patients to severe skin blistering and scarring, leading to high morbidity and mortality. It is caused by mutations in the COL7A1 gene encoding type VII collagen, an essential component of the dermal-epidermal basement. While several experimental somatic cell therapies for RDEB are currently being explored, these therapies carry significant safety risks due to the use of viral vectors and the limited proliferative capacity of somatic cells. The technology to generate induced pluripotent stem cells (iPSCs) and advancements in the CRISPR/Cas9 gene editing technology hold great promise for curing debilitating diseases such as RDEB. In a clinical scenario, cells can be collected from an RDEB patient. The RDEB-causing mutation can be corrected using Cas9 gene editing. The corrected RDEB iPSCs can be then differentiated into skin cells and transplanted back to the same patient in need of treatment. While promising for clinical applications, CRISPR/Cas9 also suffers from significant flaws because of its low efficiency and off-target cleavage activity. To increase the accuracy and efficiency of COL7A1 correction in RDEB iPSCs, different variants of Cas9, such as two high fidelity Cas9 variants, HypaCas9 and eSpCas9, can be used in a side-by-side comparison with the prime editing technology. The latter utilizes Cas9 fused with reverse transcriptase. We have previously reprogrammed RDEB patient fibroblasts into iPSCs and generated genetically corrected RDEB iPSCs using wild type Cas9. Utilizing high fidelity Cas9 variants and the prime editing system, we are generating additional genetically corrected RDEB iPSC lines to compare the off-target and on-target activity of each gene editing strategy. This will be achieved by performing the whole genome circle sequencing procedure first to identify potential off-target cleavage sites of Cas9 followed by whole genome sequencing of RDEB iPSC lines corrected using either prime editing or different Cas9 variants. Identification of the most efficient and accurate gene editing system for correcting the COL7A1 mutations will make the iPSC-based therapy for RDEB significantly safer and will accelerate its clinical translation.

Keywords: CRISPR/Cas9, COL7A1, RDEB

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

TOPIC: HEMATOPOIETIC SYSTEM

209

HEMATOPOIETIC RECONSTITUTION AND LINEAGE COMMITMENT IN HSC GT PATIENTS ARE INFLUENCED BY THE DISEASE BACKGROUND

Calabria, Andrea - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milano, Italy*
Spinozzi, Giulio - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milano, Italy*
Benedicenti, Fabrizio - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Cesana, Daniela - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Pais, Giulia - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Scala, Serena - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Lidonnici, Maria Rosa - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Scaramuzza, Samantha - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Albertini, Alessandra - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Esposito, Simona - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Calbi, Valeria - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Fumagalli, Francesca - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Ferrua, Francesca - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Marktel, Sara - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Naldini, Luigi - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Ferrari, Giuliana - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Aiuti, Alessandro - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*
Eugenio, Montini - *SR-TIGET San Raffaele Telethon Institute for Gene Therapy, IRCCS Ospedale San Raffaele, Milan, Italy*

Abstract: Lentiviral vector (LV)-based hematopoietic stem cell (HSC) gene therapy (GT) applications have shown clear efficacy and safety profiles for the treatment for a variety of genetic diseases, confirmed by high throughput clonal tracking using vector integration sites (IS). Several factors may impact on the kinetics of hematopoietic reconstitution (HR), lineage specification, safety and efficacy. To dissect the impact of disease background and treatment age on the HR after transplantation, we studied the clonal reconstitution and multilineage potential over time in 48 patients enrolled in 3 different HSC-GT clinical trials related to distinct contexts: a neurodegenerative lysosomal storage disorder (metachromatic leukodystrophy, MLD), a primary immune deficiency (Wiskott-Aldrich syndrome, WAS), and a hemoglobinopathy (β-thalassemia). Since no signs of insertional mutagen-

esis has been identified, we analyzed the clonal repertoire of >3.5 million HSPC clones to dissect the HSC activity/commitment over time. All patients showed a similar pattern of HR over time characterized by an early fluctuating period of 6-9 months after GT followed by stabilization (>24 months). The estimated number of active HSPCs within 6 months was ~80,000, decreasing and stabilizing thereafter to ~11,000, highlighting the relevant role of short lived progenitors (committed myeloid cells) in sustaining the early phases of HR and exhausting after 9 months. In all studies, committed short living HSPC were significantly less represented in the pool of long lasting clones, confirming the limited life-span of infused committed clones. Although in all studies multilineage clones were highly represented throughout patient's life, the commitment of HSPCs changed according to the disease background. In MLD patients, myeloid-committed clones increased over time (at 40%), whereas in WAS T-cell lineage committed clones increased over time (at 30%) as expected by the selective advantage in T cells, and in β-Thal patients myeloid and erythroid committed clones showed an increase. Our data suggest that the disease condition influences the proportion and the type of lineage-committed cells over time, and that the engrafted HSPC pools dynamically respond to the disease-specific physiopathology to restore normal hematopoiesis.

Funding Source: Fondazione Telethon
Keywords: Hematopoietic stem cell Gene Therapy, Clonal tracking, Stem cell activity and commitment

TOPIC: MUSCULOSKELETAL

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A PUTATIVE SMALL MOLECULE MEDIATED INHIBITION OF TGF BETA 1 PREVENTS FIBROSIS IN AN ANAL SPHINCTER INJURY MODEL

Kumar, Ashis - *Centre for Stem Cell Research, Christian Medical College, Vellore, India*
Coelho, Victor - *General Surgery, Christian Medical College, Vellore, India*
Gera, Sahil Kumar - *Bioengineering, Christian Medical College, Vellore, India*
Devashayam, Suresh - *Bioengineering, Christian Medical College, Vellore, India*
Nayak, Sukria - *General Surgery, Christian Medical College, Vellore, India*
Madhuri, Vrisha - *Paediatric Orthopaedics, Christian Medical College, Vellore, India*

Abstract: Wound healing and regeneration of injured tissues are delayed by trans differentiation of fibroblast to myofibroblast because of prolonged inflammation associated elevation of TGF beta 1 signaling. Our hypothesis is to test the sustained delivery of a suitable small molecule targeting TGF beta 1 signaling and inhibit the trans differentiation of fibroblast and accumulation of extracellular matrix in an anal sphincter injury model in rat. Muscle derived stem cells (MDSC) and muscle resident fibroblast were isolated from gastrocnemius muscle, of Sprague Dawley rats (SD). Anal sphincter injury was created in 40 SD rats (10 in each group) and validated by sphincter force assessment device. Small molecule (SM) was tested using a invitro fibroblast culture model for cell migration, cell proliferation, cytotoxicity and fibrosis markers. MDSC and the SM were delivered at the site of defect using a fibrin hydrogel carrier in group 3 and group 4, while the group 2 received the carrier and group 1 served as untreated control. Invivo experiments were followed up for 3 months with periodic sphincter force assessment, immunohistology and expression studies in the tissue samples at the end of follow up. Sphincter contractility at pre and post-injury follow up shows significant amount of force reduction in the injury model confirming the loss of sphincter function. The histology revealed complete (100%) fibrosis at the sight of injury, with no evidence of muscle regeneration in untreated control group, while MDSC transplanted animals had moderate (60 to 80%) fibrosis with new muscle formation (15-25%) at the site of implant. SM treated fibroblast and myofibroblast shows reduction in smooth muscle actin expression and increased cell migration in the scratch assay compared to the untreated control and TGF beta 1 treated fibroblast. Animals treated with sustained delivery of SM showed significant reduction in the amount of fibrosis with absence of collagen accumulation and an increase in new muscle formation. Our results demonstrate the proof of concept for potential reversibility of the myofibroblast in early muscle fibrosis in an anal sphincter injury model. Further preclinical studies are required to identify the signaling molecule downstream TGF-b1 receptor as potential targets.

Keywords: Myofibroblast and TGF beta signaling, Muscle derived stem cells, Fibrosis

TOPIC: NEURAL

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PRELIMINARY SAFETY AND TOLERABILITY FINDINGS FROM ON ONGOING PHASE 1/2 STUDY OF A SINGLE DOSE OF INTRAVENOUS (IV) IMS001 IN SUBJECTS WITH MULTIPLE SCLEROSIS (MS)

Wang, Xiaofang - *Research and Development, ImStem Biotechnology, Inc., Farmington, CT, USA*
Thrower, Ben - *Multiple Sclerosis Institute, Shepherd Center, Atlanta, GA, USA*
Foely, John - *Rocky Mountain MS Clinic, Rocky Mountain MS Clinic, Salt Lake City, UT, USA*
Ionete, Carolina - *MS Center, UMass Memorial Health Care, Worcester, MA, USA*
Garcia, Dunia - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Peng, Tao - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Shen, Hua - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Liu, Xiufang - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Libera, Agata - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Song, Li - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*
Kim, Richard - *Research and Department, ImStem Biotechnology, Inc., Farmington, CT, USA*

Abstract: IMS001 is an allogeneic, off-the-shelf human embryonic stem cell-derived mesenchymal stem cell (hESC-MSC) investigational drug product that is exclusively manufactured by ImStem Biotechnology. It is derived through a trophoblast intermediate stage; hence also known as T-MSC. Preclinically, it has demonstrated efficacy in an experimental autoimmune encephalomyelitis (EAE) model through immune regulation and blood-brain-barrier (BBB) stabilization. IMS001 is believed to be the first such

cellular product to have received investigational new drug (IND) application clearance to proceed in patients with multiple sclerosis (MS). IMS001-01 (NCT04956744) is a phase 1/2 dose-escalating, open-label study to evaluate the safety, tolerability, and exploratory efficacy of single dose of intravenous IMS001. The study population is open to patients with relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), or primary progressive MS (PPMS) with inadequate response to at least 2 prior disease modifying treatments (DMTs). The study aims to enroll about 20-30 subjects in 2-3 cohorts with 10 subjects per cohort at 3-4 sites in the United States. Primary objective of the study is to determine safety, tolerability, and dose-limiting toxicities (DLTs) and to determine the optimal dose regimen for subsequent phases of development. The main treatment phase of investigation lasts 12 months with an additional 4-years of long-term safety follow-up. 2 sites in the US, the Shepherd Center in Atlanta and the Rocky Mountain MS Clinic in Salt Lake City, have been initiated with 1 additional site pending ethics committee approval at the University of Massachusetts in Worcester. 3 patients have been successfully dosed. Although limited by small sample size, dosing has been safe and well tolerated to date with no deaths or serious adverse events. Adverse events observed to date have been generally mild to moderate in severity. More updated safety and tolerability data will be presented. As a first-in-class, allogeneic, off-the-shelf cellular therapy, IMS001-01 aims to generate safety, tolerability, and exploratory efficacy data that may support subsequent stages of development.

Funding Source: Connecticut Regenerative Medicine Research Fund #13-SCDIS-ISB-01 ImStem Biotechnology, Inc. Private Funding
Keywords: mesenchymal stem cells, multiple sclerosis, clinical trialsTopic: Pluripotent Stem Cells

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SEARCH FOR AND CHARACTERIZATION OF SUSTAINED TRANSGENE EXPRESSION LOCI (STEL)

Soh, Chew-Li - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
McAuliffe, Conor - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Wilkinson, Dan - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Burnett, Benjamin - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Forton-Juarez, Ana - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Kosmyna, Brian - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Lafaille, Fabien - *Process Development, BlueRock Therapeutics, New York, NY, USA*
Mann, Shannon - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Bendriem, Raphael - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Lee, Jeehoon - *Cardiology, BlueRock Therapeutics, Toronto, ON, Canada*
Laronde, Sarah - *Cardiology, BlueRock Therapeutics, Toronto, ON, Canada*
Gomez-Aristizabal, Alejandro - *Immunology, BlueRock Therapeutics, Toronto, ON, Canada*
Umana, Juan Mauricio - *Immunology, BlueRock Therapeutics, Toronto, ON, Canada*
Douvaras, Panagiotis - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Sun, Bruce - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Srinivas, Maya - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Ebel, Mark - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*
Tomishima, Mark - *Platform Discovery, BlueRock Therapeutics, New York, NY, USA*

Abstract: Therapeutic cell products derived from human pluripotent stem cells (hPSCs) may be genetically altered to enhance or modify cellular function through the stable integration of a transgene into the genome. However, stable long-term transgene expression within a cell remains a challenge in the field because an introduced transgene may be subject to DNA methylation and histone modifications during hPSC differentiation and subsequent maturation that result in chromatin remodeling to cause transgene silencing. There thus remains a need to identify transgene integration sites that permit sustained transgene expression in hPSCs and their derivatives. In this study, we appraised single cell RNA sequencing (scRNAseq) data collected from hPSCs and their differentiation derivatives including dopaminergic neurons, ventricular cardiomyocytes and myeloid progenitors to perform a site survey for STEL candidates. The data were collected from 267,058 single cell transcriptomes. Transcripts were ranked by prevalence, uniformity and level of expression. We hypothesized that we could drive high level sustained transgene expression from the endogenous gene structures of these highly ranked STEL candidates. Two putative STEL sites, GAPDH and RPL13A, were selected for testing of payload expression. CRISPR/Cas9 was used to insert an EGFP test cargo that was linked in-frame to the 3' UTR of the endogenous STEL gene via a 2A peptide. Clonally derived GAPDH::EGFP and RPL13A::EGFP hPSC lines robustly and stably expressed EGFP over multiple passages in culture, and after lineage-directed differentiation towards either a dopaminergic, cardiomyocyte, macrophage or microglia cell fate. All cells that were targeted at the STEL locus expressed the transgene, without evidence of silencing. These loci are currently being evaluated for the expression of transgenes that carry various modalities in different therapeutic settings including the expression of functional proteins and enzymes, secreted antibodies and secreted cytokines. Akin to our EGFP reporter lines, we observed similar robust, stable and ubiquitous expression of these various biological cargo confirming the reliability of our STEL platform for transgene expression.

Keywords: Transgene expression, Gene targeting in hPSCs, Genome engineering

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ENGINEERING THE IN VITRO ORGANOID MICROENVIRONMENT FOR POSITIVE SELECTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED, CLASS I-NULL, ANTIGEN SPECIFIC T CELLS

Chang, Patrick C. - *Molecular Biology Institute, University of California, Los Angeles, CA, USA*
Zampieri, Alexandre - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Yuan, Xuegang - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA),*

CA, USA
Towns, Chloe - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Yoo, Sang Pil - *Molecular Biology Institute, University of California, Los Angeles (UCLA), CA, USA*
Tsai, Steven - *Hematology-Oncology, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Zhu, Yuhua - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Montel-Hagen, Amelie - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Seet, Christopher - *Hematology-Oncology, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*
Crooks, Gay - *Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles (UCLA), CA, USA*

Abstract: While T cell-based immunotherapies using cells expressing antigen-specific receptors (CARs or TCRs) have produced promising clinical responses, current approaches are limited to autologous T cells due to the risk of graft-versus-host disease (GvHD) from allogeneic T cells through endogenous TCR expression and rejection through MHC incompatibility. Human pluripotent stem cells (hPSCs) have the potential to address these challenges as they are an infinitely self-renewing source of hematopoietic cells, and are amenable to gene-editing approaches to address alloreactivity. Here, we report the development of Class I MHC-null, antigen-specific, naïve CD8 T cells from gene-edited hPSCs using the "Artificial Thymic Organoid" (ATO) system previously developed by our lab, which induces highly efficient and reproducible differentiation of naïve T cells from hPSC sources. To prevent alloreactive TCR generation, we deleted both of the recombination activation genes (RAG1 and RAG2) via CRISPR/Cas9 to generate RAG1-/-RAG2-/- double knockout (DKO) hPSCs. Subsequently, DKO hPSCs were edited to eliminate surface expression of Class I MHC by knocking out Beta-2-microglobulin (B2M) via CRISPR/Cas9 to generate RAG1-/-RAG2-/-B2M-/- triple knockout (TKO) hPSCs. As predicted, T cell development from DKO and TKO hPSCs was arrested due to the loss of endogenous TCRs, and T cell precursors generated from TKO hPSCs were Class I MHC-null. To support positive selection in the absence of endogenously rearranged TCRs, DKO and TKO hPSCs were transduced with lentivirus to express the HLA-A*0201-restricted 1G4 TCR recognizing the tumor antigen NYESO. Naïve, antigen-restricted T cells were exclusively generated from 1G4 TCR-transduced DKO hPSCs that endogenously expressed HLA-A*0201, demonstrating that positive selection in the ATO system is restricted to the cognate MHC of the 1G4 TCR. In order to generate positively selected naïve T cells from TKO hPSCs, the stromal component of the ATO system was engineered to provide the cognate MHC for the 1G4 TCR, HLA-A*0201 as well as human B2M. Functional and transcriptional characterization demonstrated that TKO-NYESO TCR-engineered T cells have a similar phenotype and cytokine release profile to unedited T cells, and improved antigen-specific cytotoxicity in vivo.

Funding Source: NIH/NCI: F31CA239555 CIRM: DISC2-10134 Pluto Immunotherapeutics Inc.
Keywords: T cell development, Human Pluripotent Stem Cells, Immunotherapy

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DESIGN OF ANIMAL COMPONENT FREE (ACF) AND CHEMICALLY DEFINED (CD) MEDIUM FOR HPSC IN MONOLAYER AND 3D SUSPENSION CULTURE SYSTEMS

Daniliuc, Sharon - *Research and Development, BI Sartorious, Kibbutz Beit-Haemek, Israel*
Miropolski, Yuliya-Yael - *Research and Development, Biological Industries Sartorius, Kibbutz Beit-Haemek, Israel*
Genser-Nir, Mira - *Research and Development, Biological Industries Sartorius, Kibbutz Beit-Haemek, Israel*
Malkah, Natali - *Research and Development, Biological Industries Sartorius, Kibbutz Beit-Haemek, Israel*
Fiorentini, David - *Research and Development, Biological Industries Sartorius, Kibbutz Beit-Haemek, Israel*

Abstract: Human pluripotent stem cells (hPSC), are able to differentiate into the three germ layers of the human embryo, and are established 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 as well as proceeding toward desired differentiation. The quality of culture medium and its performance are particularly crucial regarding therapeutic applications, since hPSC properties can significantly be 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 infectious agents transmission and immune rejection of the transplanted cells. To date, there is no efficient ACF and CD medium for 2D monolayer and 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 generating quality-assured cells. The present study describes the development of ACF and CD medium suitable for hPSC expansion using recombinant matrices in 2D culture and aggregates in suspension dynamic culture. Results show that ACF and CD medium enables high proliferation rate of hPSC, while maintaining high pluripotency marker expression and stable karyotype.

Keywords: Suspension culture, Animal-component free, Chemically defined

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

915

DEPOSIT AND DISTRIBUTION OF HUMAN PLURIPOTENT STEM CELL LINES IN THE FRAME OF THE SPANISH NATIONAL STEM CELL BANK FROM THE ISO 20387 PERSPECTIVE

Catalina Carmona, Purificación - *Genetic Quality Control and Biomarkers, Andalusian Public Health System Biobank, Granada, Spain*
López Caraballo, Lidia - *Quality Control, Andalusian Public Health System Biobank the Central Node, Granada, Spain*
Aguilar Quesada, Rocío - *Genetic Quality Control and Biomarkers, Andalusian Public Health System Biobank the Central Node, Granada, Spain*

Abstract: There is a legal obligation to register and deposit in the Spanish National Stem Cell Bank all the human pluripotent stem cell lines (hPSCs) generated in Spain, guaranteeing their preservation and hence their availability and distribution for biomedical research. When a cell line is receipted in the Andalusian Public Health System Biobank, as a Node of this National Bank, Mycoplasma test is performed, cell identity is checked by genetic fingerprinting and chromosomal status by karyotype, before expansion of the cells to generate a stock available for distribution. The Andalusian Biobank has these operations included in its Quality Management System certified according to ISO 9001:2015; however, it would be desirable to comply with ISO 20387 Biotechnology – Biobanking – General requirements for biobanking, a document containing requirements to demonstrate competent biobank operations. In order to confirm if biobank operations related with deposit, testing, storage and distribution of hPSCs would be competent from the ISO 20387 perspective, analysis of the workflow and procedures covering life cycle applied to this biological material and associated data has been developed. Scope of biobanking operations has been established and procedures and documented information involved in the workflow have been identified and revised to ensure compliance with relevant requirements. When it has been necessary for compliance with ISO 20387, new actions, documents or records has been designed for critical equipment, personnel, quality controls or risks involved in the processes of deposit, testing, storage and distribution of hPSCs. The analysis performed give answer to the Option B of Clause 8 of ISO 20387 about quality management system requirements, to operate the Andalusian Biobank in accordance with both ISO 9001 and ISO 20387 international standard, which was developed to promote confidence in biobanking institutions and procedures, establishing requirements for biological material and data quality that demonstrate the organization competence in proper handling, traceability, and long-term preservation of samples.

Funding Source: This work has been funded by CONSEJERÍA DE SALUD Y FAMILIAS, JUNTA DE ANDALUCÍA; and INSTITUTO DE SALUD CARLOS III, grant number PT20/00065, co-financed by FEDER.
Keywords: Spanish National Stem Cell Bank, Quality Management System certified, ISO 9001 and ISO 20387

TOPIC: HEMATOPOIETIC SYSTEM

917

AN ENGINEERED SERUM- AND FEEDER CELL-FREE SYSTEM FOR THE EFFICIENT GENERATION OF CD19+ B CELLS FROM HUMAN STEM CELLS

Golubeva, Diana - *Research and Development/Immune Cell Culture, STEMCELL Technologies, Vancouver, BC, Canada*
Brauer, Patrick - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Babic, Sandra - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Van Eyk, Jessica - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Wang, Tinya - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Kuo, Grace - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Le Fevre, Tim - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Kokaji, Andy - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Tabatabaei-Zavareh, Nooshin - *Research and Development/Immune Cell Culture, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: B cells play a critical part in adaptive immunity, and produce long-lived plasma cells (LLPCs) that provide decades-long protection. Although LLPCs exhibit great longevity, they lack the capability to proliferate, which combined with inefficient in vitro generation of their B cell precursors—particularly in serum- and feeder cell-free conditions—is a major obstacle in their development as a cell therapeutic tool. The use of pluripotent stem cells (PSCs) is advantageous, as it can be combined with gene-editing to enable the generation of universal B, and plasma cells with capabilities such as immune evasion, engineered pathogen-specific antibodies, and safety switches for adoptive cell transfer. We developed a serum- and feeder cell-free culture system for generating B cells from human umbilical cord blood (CB)- and PSC-derived hematopoietic stem and/or progenitor cells (HSPCs). CB HSPCs were cultured in StemSpanTM SFEM II, sequentially supplemented with a progenitor supplement for 2 weeks, a B cell specification supplement for 2 weeks, and a B cell maturation supplement for 1 week. After 5 weeks, CD19+ B cells were produced with a frequency of 66 ± 3.2% (mean ± SEM, n = 12) and a yield of 336 ± 109 cells per input CD34+ cell. CD19+IgM+ B cells were produced with a frequency of 39 ± 4.7% and a yield of 194 ± 64 cells per input CD34+ cell. A subset of CB-derived B cells was composed of IgM antibody-secreting cells (ASCs), as confirmed by ELISpot assay, with an average ASC yield of 16 ± 5.0 cells per input CD34+ cell (n = 9). PSC-derived HSPCs were cultured similarly to CB-derived cells for 4 weeks on a chemically defined matrix to generate CD19+CD10+ cells with a mean frequency of 8% when gated on CD45+CD33- (range: 0 - 24%, n = 6). The resulting CB- and PSC-derived cells showed elevated expression of transcription factor genes required for B cell specification, such as PAX5. V(D)J gene rearrangement of CB-derived B cells was confirmed by the κ-deleting recombination excision circle (KREC) assay. This novel culture system robustly generates CD19+ B cells

and ASCs from primary CB-derived HSPCs and is the first report of a serum- and feeder cell-free system for the differentiation of PSC-derived HSPCs to B cells. This work enables further B lineage-specific research and provides a path to in vitro generation of LLPCs for clinical applications.

Keywords: B cells, hematopoietic stem and/or progenitor cells, human pluripotent stem cells

TOPIC: MUSCULOSKELETAL

919

TRACKING OF MUSCULOSKELETAL CELL THERAPIES: DEVELOPING A NOVEL GENE REPORTER SYSTEM

Seah, Matthew - *Department of Surgery, University of Cambridge, UK*
Birch, Mark - *Department of Surgery, University of Cambridge, UK*

Abstract: The ability to track cell therapies in animal models can increase our understanding of their localisation and function, in particular, mesenchymal stromal cells (MSCs) as a cell therapy in osteoarthritis research. However, traditional cell-tracking techniques are mainly histological which do not allow dynamic evaluation of the cell therapy, and requires large numbers of experimental animals. Here we describe a novel dual-modality gene reporter, based on the organic anion transporting protein Oatp1a1, which can be used in a musculoskeletal context. Oatp1a1 mediates uptake of the clinically approved, Gd 3+-based MRI contrast agent, as well as the radionuclide 111In. Following transfection with a lentiviral vector, cells which express Oatp1a1 can be tracked with both magnetic resonance imaging (MRI) and radionuclide imaging, combining the spatial resolution of MRI with the sensitivity of radionuclide imaging. Several assays, including cell migration and differentiation potential assays, were used to investigate the effect of lentiviral transfection on MSCs. We optimised the transfection of human bone marrow derived MSCs and show that their phenotype is similar to that of non-transfected cells under particular conditions, allowing us to track the Oatp1a1-expressing MSCs in vivo. These cells showed reversible and positive contrast (>7-fold signal enhancement) in T1-weighted MRI. Following injection of111In in the same experimental animals, the cells are also successfully longitudinally imaged using single photon emission computed tomography (SPECT). This reporter construct can be used for tracking implanted stromal cells, because the expression of the Oatp1a1 reporter is unaffected by cell division. The incorporation of tissue- or phenotype-specific promoters to the construct will also provide information about the differentiation state of the cells, allowing us to gain insights into their function along with their localisation. The combination of MRI and radionuclide imaging may allow whole-body screening for detection of labelled cells, followed by high-resolution and targeted imaging with MRI. We have used contrast agents licensed for human clinical use, and this technology could potentially be used in clinical research settings in the longer term.

Funding Source: MS is funded by a grant from the Wellcome Trust (PhD Programme for Clinicians)
Keywords: tracking, reporter, imaging

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

921

HTERT OVEREXPRESSION ENHANCES THE FUNCTIONAL EFFICACY OF MESENCHYMAL STROMAL CELLS

Arya, Smriti - *Research and Development, Rohto Advanced Research Hong Kong Ltd., Hong Kong*
Kobayashi, Eiji - *Research and Development, Rohto Advanced Research Hong Kong Ltd., Hong Kong*
Kurogi, Hikari - *Research and Development, Rohto Advanced Research Hong Kong Ltd., Hong Kong*

Abstract: Mesenchymal stromal Cells (MSCs) have been effectively used for regenerative therapy against several diseases as they exert various therapeutic effects. However, MSCs enter replicative senescence upon continuous expansion in vitro, and show inconsistent therapeutic response in vivo due to donor variations. Recent studies have reported that ex vivo manipulation of MSCs can improve their inherent therapeutic abilities. One such approach is to overexpress genes in MSCs in vitro. This study involves overexpression of hTERT (human telomerase Reverse Transcriptase) gene in umbilical cord derived MSCs (UC-MSCs) to study its effect on MSC functions and proliferation as it has been reported that hTERT affects numerous gene expression non-canonically thereby altering MSC properties. hTERT was overexpressed by two methods. Firstly, hTERT mRNA was transfected to UC-MSCs using liposomes (mR-hTERT-MSCs) and in second method, hTERT gene was delivered by lentiviral transduction to generate immortalized UC-MSCs (im-hTERT-MSCs). Both mR-hTERT MSCs and im-hTERT MSCs presented prolonged proliferation along with decreased senescence at higher passages compared to un-transfected cells and fulfilled the minimal criteria for MSCs. Notably, both MSCs showed an increased migration rate, even in the absence of chemoattractant. The secretome from both MSCs showed an improved angiogenesis ability, implying that it can be further expanded to a cell-free therapy. In mR-hTERT-MSCs, hTERT expression was not detectable after 4 passages, indicating that mRNA is transiently active, and the risk of tumorigenesis is significantly less, compared to the immortalized MSCs with constitutive hTERT expression. Hence, this study demonstrates that hTERT modification, especially the hTERT mRNA, not only provides stable MSC supply, but also presents improved therapeutic efficacy in their secretome, contributing to the development of advanced MSC based therapies in future.

Keywords: MSC, mRNA, hTERT

POSTER SESSION III: ODD

3:00 PM – 4:00 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

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TOMATIDINE -STIMULATED MATURATION OF HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES FOR MODELING MITOCHONDRIAL DYSFUNCTION

Kim, Yeseul - *Physiology, Pusan National University, Yangsan, Korea*
Yoon, Jungwon - *Physiology, Pusan National University, Yangsan, Korea*
Kim, Dasol - *Physiology, Pusan National University, Yangsan, Korea*
Kim, Deok-Ho - *Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA*
Kim, Jae Ho - *Physiology, Pusan National University, Yangsan, Korea*

Abstract: Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) have been reported to exhibit immature embryonic or fetal cardiomyocyte-like phenotypes. To enhance the maturation of hESC-CMs, we identified a natural steroidal alkaloid, tomatidine, as a new substance that stimulates the maturation of hESC-CMs. Treatment of human embryonic stem cells with tomatidine during cardiomyocyte differentiation stimulated the expression of several cardiomyocyte-specific markers and increased the density of T-tubules. Furthermore, tomatidine treatment augmented the number and size of mitochondria and enhanced the formation of mitochondrial lamellar cristae. Tomatidine treatment stimulated mitochondrial functions, including mitochondrial membrane potential, oxidative phosphorylation, and ATP production, in hESC-CMs. Tomatidine-treated hESC-CMs were more sensitive to doxorubicin-induced cardiotoxicity than the control cells. In conclusion, the present study suggests that tomatidine promotes the differentiation of stem cells to adult cardiomyocytes by accelerating mitochondrial biogenesis and maturation and that tomatidine-treated mature hESC-CMs can be used for cardiotoxicity screening and cardiac disease modeling.

Funding Source: This research was supported by research grants (NRF-2015R1A5A2009656 and NRF-2020R1A2C2011654) from the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology.
Keywords: maturation, cardiomyocyte, mitochondria

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MICROPATTERNED ORGANOIDS ENABLE MODELING OF THE EARLIEST STAGES OF HUMAN CARDIAC VASCULARIZATION

Abilez, Oscar - *Cardiovascular Institute, Stanford University, Stanford, CA, USA*
Yang, Huaxiao - *Department of Biomedical Engineering, University of North Texas, Denton, TX, USA*
Wilson, Kitchener - *Executive, Rosebud Biosciences, Orinda, CA, USA*
Lyall, Evan - *Executive, Rosebud Biosciences, Orinda, CA, USA*

Abstract: Although model organisms have provided insight into the earliest stages of cardiac vascularization, we know very little about this process in humans due to ethical restrictions and the technical difficulty of obtaining embryos at very early developmental stages. Here we show that spatially micropatterned human pluripotent stem cells (hPSCs) enable in vitro modeling of the earliest stages of cardiac vascularization, corresponding to the first three weeks of in vivo human development (Carnegie Stages 9-10). Using hPSC fluorescent reporter lines, we prospectively create 2D and 3D cardiac vascularized organoids (cVOs) by identifying a combination of growth factors that simultaneously give rise to a spatially organized and branched vascular network within endocardial, myocardial, epicardial, and progenitor cells, along with numerous extracellular matrix (ECM) proteins. Using single-cell RNA-sequencing (scRNA-seq), we show that the cellular composition of cVOs resembles that of a 6.5 post-conception week (PCW) human heart (Carnegie Stages 19-20). Furthermore, we use machine learning to characterize differences in CM and EC formation. We find that NOTCH, BMP, and VEGF pathways are upregulated in cVOs and inhibition of these pathways disrupts vascularization. Finally, using the same vascular-inducing factors to create cVOs, we produce hepatic vascularized organoids (hVOs). This suggests that there is a conserved developmental program for creating vasculature within different organ systems. Our model provides an in vitro reference for fundamental understanding of how vasculature co-develops and self-organizes in the context of multicellular systems.

Funding Source: NIH K01 HL130608 (OJA); AHA Postdoctoral Fellowship 18POST34030106 (HY); NIH K08 HL119251 (KDW); Stanford MCHRI TIP Grant (OJA); Stanford CVI Seed Grant (OJA, HY); Stanford Bio-X Program (OJA)
Keywords: human cardiac vascularization, micropatterned hPSC organoid, bioengineering early development

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IMPROVED DRUG RESPONSE PREDICTION USING SCALABLE 3D HIPSC-DERIVED CARDIAC MICROTISSUES

De Korte, Tessa - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
François, Louise - *Discovery Technology, Ncardia, Leiden, Netherlands*
Matsa, Elena - *Discovery Technology, Ncardia, Leiden, Netherlands*
Mol, Mervyn - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Braam, Stefan - *Ncardia, Leiden, Netherlands*
Mummery, Christine - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Davis, Richard - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*

Abstract: The sensitivity and accuracy of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as disease models would be improved further by generating models that more closely resemble the adult heart. Various 3D cardiac models have been developed that bridge the limitations of conventional 2D models, but show batch-to-batch variations and require high cell numbers along with complex equipment. As a result, this restricts their suitability for use in high throughput screenings. Previously, we have shown that a tri-cellular combination of

cardiomyocytes, endothelial cells, and fibroblasts in 3D cardiac microtissues (MTs) enhances maturation. We were therefore interested to determine whether these MTs are scalable and can improve drug response prediction and cardiac disease modelling. We have established such strategies to generate MTs, as well as procedures to evaluate the functionally-matured cardiomyocytes. The differentiation protocols for all 3 cell types are initially small molecule-based, circumventing the need to use cytokines for the first induction step. To assess the functionality of MTs, we developed an assay to measure calcium transients of MTs loaded with a calcium-sensitive fluorescent dye in high throughput (384 well plates). To test the predictivity of MTs compared to conventional 2D monolayers, we performed a blind screen of 12 reference drugs with each having a specific mechanism of action that affects contractility. Some challenging drugs such as PDE3 inhibitors were wrongly classified using 2D models while correctly identified in 3D MTs. Overall, the blind screen in MTs resulted in 92% of drugs being accurately classified based on the effects on 7 calcium transient-related parameters. We have also established procedures to automate the MT formation process. Using robotics, a 384 well plate of MTs can be formed in < 2 minutes, and show similar morphology and functionality as manually seeded MTs. Overall, our results indicate that 3D MTs are scalable, amenable to automation and show improved predictivity of drug responses. Consequently, 3D cardiac MTs hold great potential for cardiac disease modeling and high throughput screenings.

Funding Source: NovoNordisk Foundation (ReNEW; #NNF21CC0073729); ERC-StG (STEMCARDIORISK; #638030); NWO-funded VIDI fellowship (ILLUMINATE; #91715303); Gravitation project (NOCI; #024.003.001)
Keywords: hiPSC, high throughput screening, 3D cardiac models

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HUTCHINSON-GILFORD PROGERIA SYNDROME LAMINOPATHY CAUSES CARDIAC ALTERATIONS IN IPSC-DERIVED CARDIOMYOCYTES

Mesquita, Fernanda - *Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA*
Morrissey, Jacquelynn - *Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA*
Curty da Costa, Ernesto - *Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA*
Lim, Jing Ming - *Biochemistry Department, University of Houston, College of Natural Sciences and Mathematics, Houston, TX, USA*
Horton, Renita - *Biomedical Engineering Department, University of Houston, Cullen College of Engineering, Houston, TX, USA*
Hochman-Mendez, Camila - *Regenerative Medicine Research, Texas Heart Institute, Houston, TX, USA*

Abstract: Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare premature aging disease caused by a mutant lamin A resulting in nuclear accumulation of progerin. Death commonly occurs from myocardial infarction and stroke during teenage years. Studies using HGPS-derived induced pluripotent stem cell (iPSC) lines are critical to elucidating HGPS' deadly cardiovascular pathophysiology. Although progerin-induced alterations have been well described for endothelial cells in in vitro studies, knowledge is severely lacking with regard to the cardiac cell biology of the disease. Here we differentiated cardiomyocytes (CMs) from HGPS-derived iPSCs and characterized their cardiac phenotype. The HGPS-CMs presented characteristics of laminopathy (higher expression of progerin and abnormal nuclear morphology), though the cardiac differentiation was not impacted by the mutation. Premature aging (shorter telomere length, higher ROS level, and increased expression of p21 Waf1/Cip1 marker) was observed in HGPS-CM compared to control. HGPS-CMs exhibited notable differences in electrophysiological properties versus CTRL-CMs, including a decrease in beating rate and amplitude, and less responsiveness to an L/T-type Ca blocker and β-adrenergic agonist. Both groups responded similarly to hERG blocker, supporting the qPCR findings showing differences in Ca2+-related genes and no difference in K+ -related genes. Arrhythmic cells were observed by the presence of early afterdepolarization (EAD) in HGPS-CMs. Taken together, we report successful differentiation of HGPS iPSCs to CMs where cells revealed laminopathy-induced molecular and electrical alterations. These results are a step forward to understanding effects of HGPS-specific cardiomyocyte pathology.

Keywords: Hutchinson-Gilford progeria syndrome, iPSC-derived cardiomyocytes, disease model

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GENERATION OF MULTI-CELLULAR HUMAN HEART-LIKE ORGANFOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Song, Myeongjin – *NEXEL Co., Ltd., Gangseo-gu, Seoul, Korea*
Choi, Dabin - *Department of Commercializing Stem Cell Technology, NEXEL Co., Ltd., Gangseo-gu, Seoul, Korea*
Im, Jeongsuk - *Department of Commercializing Stem Cell Technology, NEXEL Co., Ltd, Gangseo-gu, Seoul, Korea*
Woo, Dong-Hun - *Department of Commercializing Stem Cell Technology, NEXEL Co., Ltd, Gangseo-gu, Seoul, Korea*

Abstract: Cardiogenesis is a tight spatiotemporal regulation with an orchestration of diverse cell types, including cardiomyocytes, conducting tissues, and non-muscle cells. To date, many researchers have established 3D structure cardiac models from human induced pluriopotent stem cells (hiPSCs) that could mimic in vivo cardiogenesis. However, current protocols for heart organoid differentiation from hiPSCs have been generated the clumps of cardiomyocytes that do not closely represent biology of human heart. Here, we developed a modified method to differentiate self-organizing heart organoids from hiPSCs (NEXEL's heart organoids) with vascular network and extracellular matrix environment. NEXEL's heart organoids showed similar cellular distributions with the human heart, thus, the organoids involve cardiomyocytes as well as conducting cells such as smooth muscle cells and endothelial cells. Moreover, NEXEL's heart organoids exhibited physiologically matured characteristics compared to the conventional cardiac organoids, and the organoids efficiently recapitulated a myocardial infarction under heart disease environments. Therefore, this multi-cellular heart organoids not only provides a biomimetic human heart-like organoids, but also suggested a promising research method with a broad application including cardiac disease modeling.

Keywords: Human induced pluripotent stem cells, Cardiac organoid, Cardiomyocyte

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CHARACTERIZATION OF A NOVEL TRUNCATION VARIANT OF TBL1X IDENTIFIED IN DILATED CARDIOMYOPATHY

Kim, Hyeong-Jin - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seodaemun-gu, Korea*
Lee, Sun-Ho - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Choi, Hyo-Kyung - *Division of Nutrition and Metabolism Research Group, Korea Food Research Institute, Seoul, Korea*
Oh, Jaewon - *Division of Cardiology, Severance Cardiovascular Hospital, Cardiovascular Research Institute, Seoul, Korea*
Kang, Seok-Min - *Division of Cardiology, Severance Cardiovascular Hospital, Cardiovascular Research Institute, Seoul, Korea*
Yoon, Ho-Geun - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Park, Sahng Wook - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*
Lee, Seung-Hyun - *Biochemistry and Molecular Biology, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Although dilated cardiomyopathy (DCM) is a heart disease that causes heart failure and is a major cause of heart transplantation, the exact pathogenesis of DCM is not yet clear. In order to investigate the genetic causes of DCM, whole-exome sequencing analyses were performed on a DCM patient and non-DCM family members of the DCM patient. As a result, we identified a novel homozygous nonsense mutation in exon 15 of the transducin β-like 1 X-linked protein (TBL1X). Our study found that the overexpressed TBL1X truncated variant (TBL1Xtv) was significantly reduced in hiPSCs (human induced pluripotent stem cells)-derived cardiomyocytes compared to the wild-type, although there was no difference in mRNA levels. In addition, inhibition of proteasome activity by using MG132 effectively rescued the decreased TBL1Xtv protein level. These results suggest that massive TBL1Xtv degradation depends on proteasome activity. Using western blot, we investigated whether post-translational modifications control protein stability. We found that there was no difference between wild-type and phospho-mimetic TBL1X (S470/474D) protein expression levels. However, SUMO-TBL1X protein showed increased protein stability. The sumoylation of TBL1X enhanced the interaction between beta-catenin and TBL1X, leading to activation of Wnt target genes. TBL1Xtv failed to increase LiCl-induced Wnt signaling in contrast to wild-type. In addition, the level of TBL1X protein expression in DCM patient heart tissue was significantly reduced compared to normal heart tissue. Thus, the decreased expression level of TBL1X by the truncating variant may affect the development of DCM. Next, we introduced TBL1X truncated variant hiPSC-CMs. A patient sequence-specific sgRNA was designed and cloned into the pSpCas9(BB) vector, which, together with the cas9 expression, was co-transfected into a hiPSC with donor DNA. Transfected cells were selected with puromycin, and the efficiency of the base-editing was analyzed. The modified cell-derived cardiomyocyte was observed reduced cardiac function and immature phenotype compared to normal cardiomyocyte. In conclusion, we suggest that decreased TBL1X expression level with protein truncation is associated with the development of the DCM.

Funding Source: This work has supported by the National Research Foundation of Korea (NRF) grant (2022R1H1A200391911, 2019R1C1C1002334).
Keywords: TBL1, CRISPR/cas9, hiPSC-CM

TOPIC: EARLY EMBRYO

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THE ROLE OF YAP1 IN THE MOST IMPORTANT EVENT OF EVERYONE'S LIFE: GASTRULATION

Estaras, Conchi - *Cardiovascular Sciences, Temple University, Philadelphia, PA, USA*
Abraham, Elizabeth - *Cardiovascular Sciences, Temple University, Philadelphia, PA, USA*
Stronati, Eleonora - *Cardiovascular Sciences, Temple University, Philadelphia, PA, USA*
Zubillaga, Mikel - *Cardiovascular Sciences, Temple University, Philadelphia, PA, USA*

Abstract: Long-standing studies in embryos have shown that polarized extraembryonic signals generate a NODAL:SMAD2/3 gradient in the epiblast that regulates gastrulation patterning and the establishment of the anterior-posterior (A-P) axis. However, epiblast-like cells (hESCs and mESC) organize in 2D and 3D gastruloid structures in vitro in the absence of extraembryonic tissues. These observations highlight the importance of intrinsic self-organizing mechanisms in hESCs, independent of polarized extraembryonic signals. Indeed, human 3D-gastruloids display features of Carnegie-stage-9 embryos, with a high degree of organization in gene expression along the A-P axis, including a posterior-to-anterior signature of somitogenesis. The A-P pattern correlates with the organization of signaling components along the length of the gastruloid. In elongated gastruloids, BMP signals are predominantly anterior, while the expression of WNT3 and NODAL genes are restricted to the posterior end, consistent with a role of the latter in the mammalian tailbud at the onset of gastrulation. However, how the regionalization of the signaling components is achieved in the 3D-gastruloids is unknown. Our ongoing analysis suggest that the Hippo-effector YAP1 regulates the antero-posterior organization of the 3D-gastruloids by restricting the posterior expression of Nodal signaling components. Our data show that YAP1 represses Nodal activity through the recruitment of the Polycomb-repressor complex to the chromatin of Nodal genes in hESCs. Hence, in the 3D-gastruloids, YAP1 deletion lead to the anterior expansion of Nodal:Smad2/3 activity. As a consequence, the YAP1 KO-derived 3D-structures are longer than controls, suggestive of an increase in Nodal-induced mesoderm derivatives. Finally, conditional deletion of YAP1 in the mouse gastrula leads to the over specification of mesoderm precursors, mimicking the phenotype of Nodal overexpressing mutants. Our findings highlight a crucial interaction between YAP1 and NODAL signaling essential for the germ-layer formation and morphogenetic events occurring during the establishment of the body plan.

Keywords: YAP1 signaling, Nodal signaling, Axial elongation of 3D-gastruloids

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EXTRAEMBRYONIC CELLS INDUCE GASTRULATION LIKE BEHAVIOR IN HUMAN PLURIPOTENT STEM CELLS

Kong, Xiangyu - *Biosciences, Rice University, Houston, TX, USA*
Nemashkalo, Anastasiia - *B11 Division, Los Alamos National Laboratory, Los Alamos, NM, USA*
Ortiz Salazar, Miguel Angel - *Biosciences, Rice University, Houston, TX, USA*

Warmflash, Aryeh - *Biosciences, Rice University, Houston, TX, USA*

Abstract: During gastrulation, cells differentiate to the three germ layers, and the body axes are established under the control of signaling through the BMP, Wnt and Nodal pathways. Due to both ethical and technical challenges, many basic questions about human gastrulation regarding how patterns of signaling are established and interpreted remain unresolved. In the past decade, several in vitro models were developed using human pluripotent stem cells (hPSCs), such as 2D and 3D human gastruloids, and these are beginning to be used to mechanistically dissect the signaling interactions that regulate gastrulation. These models provide great flexibility for experimental manipulation and scalability and ideal conditions for imaging. However, a limitation of the current in vitro models for human gastrulation is that they are initiated by exogenously supplied signaling factors that activate the BMP or Wnt pathway, in contrast to the in vivo situation where signaling initiates in extraembryonic tissues. To understand how interactions between embryonic and extraembryonic tissues initiate processes involved in gastrulation, we developed a simple experimental system where extraembryonic and embryonic cells are juxtaposed side by side to recreate the interactions between these tissues. We found that when hPSCs are juxtaposed with amnion like cells (AMLCs), hPSCs near the border recapitulate several aspects of gastrulation including ordered differentiation to mesendodermal fates marked by BRACHYURY (BRA) and SOX17, epithelial to mesenchymal transition, and directed cell migration. We demonstrated that BMP, Wnt, and Nodal signaling were all essential for these interactions, and directly observed the patterns of activity through these pathways as well as the cell migrations that together create patterns of cell fate in time. Examining the maturation of extraembryonic tissues in this system, also demonstrated a novel differentiation route for the enigmatic extraembryonic mesoderm. These results demonstrate the potential of using juxtaposition experiments to study the native signaling events between different cell populations.

Funding Source: Simons Foundation NSF
Keywords: Gastrulation, In vitro model, extraembryonic-embryonic interaction

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ALTERED GERM LAYERS SPECIFICATION AS A CAUSE OF 22Q.11 DELETION

Stronati, Eleonora - *Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA*
Abraham, Elizabeth - *Center for Translational Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*
Zubillaga, Mikel - *Center for Translational Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*
Estaras, Conchi - *Center for Translational Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA*

Abstract: 22q.11 deletion, known as DiGeorge syndrome, is the most common microdeletion in human and is the result of a hemizigous deletion of 1.5 to 3 Mb in the chromosome 22. It has a wide spectrum of clinical presentations that affects multiple organs. Furthermore, it has been proposed that variation in maternal teratogens exposure, such as Vitamin A, can influence the incidence and severity of the disorder. Among the deleted region, several genes of interest have been identified that recapitulate just some of the 22q.11 features. Despite the efforts spent in the search for causative genes in mice, the multi-organ malformations observed in human remains unclear. We suggest the involvement of a common gestational defects as a cause of the DiGeorge syndrome. Gastrulation is an extremely sensitive period in the embryo development, in which fundamental feature of the body plan, such as three germinal layers specification and establishment of the body axis, take place and it is highly susceptible to teratogens exposure, such as Vitamin A/Retinoic Acid. Here we identify the altered gastrulation as the common developmental defects of the DiGeorge syndrome by comparing human and mouse gastrulation process. By using DiGeorge patients induced pluripotent stem cells (iPSCs) and LgDel mouse model we unveiled a defective germ layer specification due to an enhanced mesodermal layer specification and a concomitant reduction of the ectoderm. Moreover, we demonstrated an increased sensitivity to Retinoic Acid exposure both in human DiGeorge iPSCs and LgDel mice compare to healthy control. Finally, our study provides new insight in the etiology of this developmental disorder.

Keywords: 22q.11 deletion syndrome, iPSC, gastrulation

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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ENGINEERING COMPLEX ALVEOLAR ORGANOIDS TO STUDY LUNG DEVELOPMENTAL DISORDER

Pek, Nicole - *Perinatal Institute, Center for Stem Cell & Organoid Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Chen, Ya-Wen - *Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Gu, Mingxia - *Perinatal Institute, Center for Stem Cell & Organoid Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Guo, Minzhe - *Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Kotton, Darrell - *Center for Regenerative Medicine, Boston University, Boston, MA, USA*
Miao, Simon - *Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Pastrana-Gomez, Victor - *Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Rottier, Robbert - *Pediatric Surgery, Erasmus University Medical Center, Rotterdam, Netherlands*
Sun, Xin - *Pediatrics, University of California, San Diego, CA, USA*
Whitsett, Jeffrey - *Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Abstract: Disrupted pulmonary alveologenesis underlies a group of congenital lung diseases such as Alveolar Capillary Dysplasia (ACD). ACD is characterized by vascular defects such as reduced pulmonary alveolar capillaries, thickened pulmonary arteries, and misaligned pulmonary veins, which collectively contribute to respiratory distress and failure in patients. While mutations in FOXF1, a gene coding for a transcription factor critical for pulmonary vascular development, have been well-reported in ACD, how FOXF1 mutations lead to vascular malformations and abnormal alveologenesis in ACD have not been elucidated in human models. Addressing this gap in knowledge will inevitably facilitate the understanding of vascular involvements during alveologenesis and open doors to therapeutic interventions. Single-nuclei RNA sequencing of lung tissues from ACD patients revealed a significant reduction of specific lung capillary endothelial subtype- aerocytes

(aCAP). To explore the early developmental events that led to the loss of aCap in the alveolus, we generated vessel organoids (VOs) from control and ACD iPSCs harboring FOXF1 mutations. ACD VOs showed reduced expression of endothelial cell (EC) markers and abnormally elevated smooth muscle cell markers. Poorly formed vascular networks that lacked lumen structures were also observed within ACD VOs. Additionally, we observed that the number of EDNRB-expressing ECs (aCAP marker) was reduced in ACD VOs and is accompanied by lowered expression of aCAP markers. Therefore, we were able to recapitulate aCAP abnormalities uncovered by snRNA-Seq. ACD lung tissues samples also showed several pneumocyte pathologies such as disrupted alveolar type 1 (AT1) differentiation and function, which is likely contributed by vascular anomalies. Hence, we further investigated into how the vascular abnormalities caused by FOXF1 mutations impact the development of lung epithelium during alveologenesis by constructing assembloids using both vessel and lung organoids (VO-LuO). This alveolar organoid model provides a unique platform to elucidate the disrupted EC-epithelial crosstalk underlying defective alveologenesis in ACD.

Funding Source: NIH LungMAP2
Keywords: Vessel organoids, Alveologenesis, Assembloids

TOPIC: EPITHELIAL_LUNG

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VASCULARIZATION OF ALVEOLAR HUMAN LUNG ORGANOIDS DRIVES MATURATION AND LUNG INJURY INDUCED BY ENDOTOXIN AND SARS-COV-2

Syed, Abdul Qadir - *Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, IL, USA*
Malik, Asrar - *Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, IL, USA*
Rehman, Jalees - *Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, IL, USA*

Abstract: Human organoids have proven valuable for disease modeling and a deeper understanding of human organ development and function. Human lung organoids to date have focused on the differentiation of human lung epithelial subtypes, but have not addressed the role of human endothelial cells and vascularization in organoid development. Here, we describe a vascularized alveolar human lung organoid (hLO) platform generated by combining hiPSC-derived lung progenitors (LP) with hiPSC-derived endothelial progenitor cells (iEC) in a 3D matrix. Upon implantation of the in vitro generated hLO into the kidney capsules of immune deficient NOD/SCID mice, we observed formation of perfused de novo human blood vessels anastomosing with the host mouse vessels. We demonstrated an obligatory role of vascularization in promoting increased generation of alveolar type II (ATII) and type I (ATI) cells as well as activation of Wnt signaling. Organoid vascularization also increased the expression of ACE2 and protease TMPRSS2 in ATII cells, the cell surface entry receptor complex for SARS-CoV-2. The presence of human endothelial cells increased infection by SARS-CoV-2 pseudovirus and the inflammatory response induced by live SARS-CoV-2 in vascularized hLO. Endotoxemic challenge of host mice induced massive influx of mouse neutrophils into vascularized hLO, consistent with lung neutrophil sequestration seen in endotoxin-induced ARDS. Incorporation of endothelial cells into human lung organoids enables dissection of mechanisms mediating the crosstalk between lung endothelial and epithelial cells during alveolar development and inflammatory lung injury induced by endotoxin and SARS-CoV-2.

Keywords: Human lung organoid, alveolar type II cells, induced pluripotent stem cells, vascularization, lung injury, endotoxin, endothelial cells, neutrophils, SARS-CoV-2,

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STUDYING EPITHELIAL AND MESENCHYMAL COMPARTMENT DURING DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO BRONCHIAL EPITHELIUM

Nasri, Amel - *Inserm U1183, University of Montpellier, France*
Foisset, Florent - *University of Montpellier, Inserm U1183, University of Montpellier, France*
Bourdais, Carine - *University of Montpellier, Inserm U1183, University of Montpellier, France*
Ahmed, Engi - *Inserm, Montpellier, Department of Respiratory Diseases, CHU Montpellier, France*
Vachier, Isabelle - *Inserm, Montpellier, Department of Respiratory Diseases, CHU Montpellier, France*
Assou, Said - *University of Montpellier, Inserm U1183, University of Montpellier,, France*
Bourdin, Arnaud - *Inserm, Montpellier, Department of Respiratory Diseases, CHU Montpellier, France*
De Vos, John - *University of Montpellier, Inserm U1183, University of Montpellier, France*

Abstract: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide. Induced pluripotent stem cells (iPSC) provide a new approach to model lung diseases. We have generated a functional air-liquid interface bronchial epithelium from human iPSC (iALI). We focus on the characterization of mesenchymal and epithelial compartment before and after pollution exposure. To modelized COPD and study epithelium and mesenchymal crosstalk, we performed single-cell mRNA sequencing before and after pollution exposure. RT-QPCR and immunofluorescence were used to assess each step of the cell differentiation process. iALI contains all the bronchial epithelial cell subtypes such as ciliated cells, basal cells, neuroendocrine cells, club cells and goblet cells. Furthermore, a previously poorly documented EPCAM-COL1A1+DCN+ mesenchymal stromal compartment is developing below the bronchial epithelial cells of iALI. It contains several overlapping cell populations such as 1/myofibroblasts including fibromyocytes characterized by high expression of contractile genes such as CCN1 and TAGLN, 2/ a transition population expressing epithelial to mesenchymal transition transcription factor such as SNAI2 and ZEB1/2 and 3/ a highly proliferating CDK1+KIF11+ cell population. This stromal cell compartment expressed growth factors such as FGF10 while its receptor FGFR2 was expressed on the epithelial cells, suggesting that stromal cells could support iALI development. Under particle matter exposure, MUC5B was induced in epithelial cells, while CYP1B1 was induced in mesenchymal cells, reflecting an adaptive response to environmental pollution. These data provide high-resolution insights into the complexity and plasticity of the iALI and suggest that a crosstalk between epithelium and mesenchyme is essential to iALI development. Furthermore, we show

that iALI is a promising tool to model the adaptive response to environmental pollution

Keywords: human Induced Pluripotent Stem Cells (hIPSC), Chronic Obstructive Pulmonary Disease (COPD), Epithelial-Mesenchymal Transition (EMT)

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IMAGING-BASED DISEASE MODELLING OF CYSTIC FIBROSIS USING INDUCED PLURIPOTENT STEM CELLS

Yamamoto, Yuki - *HiLung Inc., Kyoto, Japan*
Kishihata, Masako - *Research and Development, HiLung Inc., Kyoto, Japan*
Mori, Yuki - *Biosciences Engineering Section, Biosciences & Pharmaceuticals, SCREEN Holdings Co.,Ltd., Kyoto, Japan*
Hisatomi, Rie - *Development Section 3, R&D Department 3, SCREEN Holdings Co.,Ltd., Kyoto, Japan*
Ito, Shunsuke - *Research and Development, HiLung Inc., Kyoto, Japan*
Miura, Takemitsu - *Development Section 3, R&D Department 3, SCREEN Holdings Co.,Ltd., Kyoto, Japan*

Abstract: Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene coding chloride ion channel. Respiratory involvement with bronchiectasis and recurrent infection is the most common cause of the deaths in CF patients. Human organoids are promising drug discovery tools for CF. Lack of established model mouse for CF emphasizes the importance of human organoids for drug discovery. In this study, we established both fluorescent and label-free functional assays of CFTR, which were useful for detection of CF-specific phenotype in vitro using airway epithelial cells differentiated from disease specific iPSC. As for a fluorescent assay, MQAE assay captured the disease-related phenotype of CF visualizing impaired efflux of chloride ion in airway epithelial cells derived from CF-iPSC with homo-delta F508 mutation. Efflux of chloride ion was partially restored by treatment with VX-809, which is one of the approved drugs for CF. Next, we established the forskolin-induced organoid swelling (FIS) assay for airway organoids without using fluorescent reagent. Label-free imaging of iPSC-derived airway organoids were performed using Cell3iMager duos. Deep learning technology was used for the process for quantification of organoid size. In normal iPSC-derived organoids, swelling was induced by treatment with forskolin over time, whereas no remarkable changes in organoids treated with vehicle control. Organoid swelling was significantly inhibited by treatment with CFTR inhibitor. Finally, we used a CF-iPSC and its isogenic control line for label-free FIS assay. Forskolin-induced organoid swelling was not observed in CF-iPSC derived airway organoids despite treatment with high concentration of forskolin, whereas approximately 150% of swelling was observed in airway organoids derived from isogenic control iPSCs. Combination of fluorescent and label-free assays using iPSC-derived airway epithelial cells will enable us to screen the promising drug candidates for CF more efficiently, as well as be applicable for personalized medicine for CF.

Keywords: cystic fibrosis, imaging assays, airway epithelial cells

TOPIC: EYE AND RETINA

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LONG-TERM RETINAL ENGRAFTMENT OF MATURE PHOTORECEPTORS GENERATED FROM TANKYRASE/PARP-INHIBITOR- REGULATED NAÏVE (TIRN) HUMAN STEM CELLS

Zimmerlin, Ludovic - *Oncology, Johns Hopkins School of Medicine, Baltimore, MD, USA*
Bhutto, Imran - *Ophtalmology, Johns Hopkins University, Baltimore, MD, USA*
Kanherkar, Riya - *Oncology, Johns Hopkins University, Baltimore, MD, USA*
Park, Tea Soon - *Oncology, Johns Hopkins University, Baltimore, MD, USA*
Barbato, Michael - *Oncology, Johns Hopkins University, Baltimore, MD, USA*
Koldobskiy, Michael - *Oncology, Johns Hopkins University, Baltimore, MD, USA*
Lutty, Gerard - *Ophtalmology, Johns Hopkins University, Baltimore, MD, USA*
Zambidis, Elias - *Oncology, Johns Hopkins University, Baltimore, MD, USA*

Abstract: Efficient differentiation of conventional, primed human pluripotent stem cells (hPSCs) into retinal photoreceptors is optimized in only a few 'permissive' lines. We recently reported a novel class of naïve epiblast-like TIRN-hPSCs with reduced interline differentiation variability, erasure of lineage priming, and that could generate embryonic progenitors with improved epigenetic plasticity and in vivo engraftment. Here, we show that TIRN-hPSCs improved retinal organoid (RO) generation. TIRN-derived RO efficiently engrafted into the subretinal space with robust differentiation into mature human photoreceptors. Isogenic primed and TIRN-hPSCs were differentiated to horse-shoe (HS)-shaped neural domains and matured to laminate-layered retinal cups (RC). Neuroepithelial specification kinetics and differentiation efficiency were quantitated at 4-20 weeks in multiple hPSC lines by RT-PCR and immunofluorescence. Transcriptional and epigenetic signatures of RO were defined at 12 weeks via RNA-Seq and CpG methylation sequencing. ROs were transplanted into the subretinal space of NOG-SCID mice, and human photoreceptor engraftment and specification was evaluated 6-10 months post-transplantation. Although several primed hPSC lines failed to differentiate, all TIRN-reverted hPSCs efficiently generated HS domains and well-differentiated ROs. Eye field-specific transcripts and progenitor markers (2-20 weeks) were detected in greater quantities in TIRN RCs. TIRN-derived ROs displayed improved maturation of rhodopsin+ photoreceptors with proper histo-architecture. NOG eyes transplanted with TIRN-derived ROs demonstrated long-term engraftment (10 months) of mature human retinal cells, and developed a full repertoire of mature rod/cone photoreceptors (e.g., rhodopsin+, L/M opsin+, recoverin+), astrocytes (GFAP+vimentin-), and Mueller cells (GFAP+vimentin+). Transcriptomic/epigenomic studies revealed an improved epigenetic plasticity in TIRN-hPSCs that was mediated by chemical PARP/Tankyrase inhibition. TIRN-hPSCs may have great impact in advancing ocular regenerative medicine by abolishing the interline variability of retinal fate specification and potentiating the long-term survival of transplanted functional photoreceptors.

Funding Source: Supported by NIH/NEI (R01EY032113; R01EY023962), NIH/NICHD (R01HD082098), Research to Prevent Blindness (Stein Innovation Award), The Maryland Stem

Cell Research Fund (2020-MSCRFD-5374), and The Lisa Dean Moseley Foundation.
Keywords: retinal organoid, PARP, naive pluripotency

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DRAM2 DEFICIENCY IN PATIENT-DERIVED RETINAL ORGANOIDS ASSOCIATES WITH LYSOSOMAL ABNORMALITIES

Tsikandelova, Rozaliya - *Bioscience Institute, Newcastle University, Newcastle Upon Tyne, UK*
Cerniauskas, Edvinas - *Biosciences Institute, Newcastle University, Newcastle, UK*
Hallam, Dean - *Biosciences Institute, Newcastle University, Newcastle, UK*
Dorgau, Birthe - *Biosciences Institute, Newcastle University, Newcastle, UK*
Yang, Chunbo - *Biosciences Institute, Newcastle University, Newcastle, UK*
Korolchuk, Viktor - *Biosciences Institute, Newcastle University, Newcastle, UK*
McKibbin, Martin - *Department of Ophthalmology, St. James's University Hospital, Leeds, UK*
Armstrong, Lyle - *Biosciences Institute, Newcastle University, Newcastle, UK*
Kurzawa-Akanbi, Marzena - *Biosciences Institute, Newcastle University, Newcastle, UK*
Lako, Majlinda - *Biosciences Institute, Newcastle University, Newcastle, UK*

Abstract: Maintenance of cellular homeostasis is achieved via the continuous recycling of damaged cellular material. Autophagy oversees this homeostatic mechanism by sequestering cargo into autophagosomes and by targeting them to lysosomes for degradation. Disease causing variants in the autophagy regulator and lysosomal membrane protein DRAM2 have been associated with an autosomal recessive form of cone-rod dystrophy presenting with early macular degeneration and secondary decline of the peripheral retina. To investigate the molecular basis of DRAM2 disease phenotype, induced pluripotent stem cells (iPSCs) derived from two DRAM2 patients and their isogenic CRISPR-corrected controls were differentiated to 3D retinal organoids. One patient was homozygous for a truncating mutation (c.140delG) which leads to a complete lack of protein due to non-sense mediated decay. The second patient was a compound heterozygote for a missense substitution at a conserved splice site (c.131G>A) and a nonsense mutation (c.494G>A). Immunofluorescence characterization of the organoids at mature stages of development showed a marked reduction in cone precursors and mature cones and rods in patient organoids. A common finding by western blot in both patients was the deficiency in lysosomal enzymes, further to a significant downregulation of late endosomal marker CD63 and differential glycosylation patterns of lysosomal membrane protein LAMP2. Patient organoids additionally showed altered expression of key lysosomal receptors LIMP2, sortilin and CDM6PR implicating DRAM2 in the transport of lysosomal hydrolases. An impairment of lysosomes was also demonstrated by the presence of aberrant looking endolysosomes and abnormal accumulation of lysosomes in patient organoids by transmission electron microscopy. Collectively, data acquired in this study outline a novel role for DRAM2 in facilitating the delivery of degradative enzymes to the lysosome.

Funding Source: Macular Society UK
Keywords: DRAM2, autophagy, lysosome

TOPIC: HEMATOPOIETIC SYSTEM

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EXOSOMES DERIVED FROM CD34+ CELLS: UNCOVERING NEW ABILITIES OF OUR TINY FRIENDS

Isaioglou, Ioannis - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Aldehaiman, Mansour - *Bioscience, KAUST, Thuwal, Saudi Arabia*
Li, Yanyan - *Bioscience, KAUST, Thuwal, Saudi Arabia*
Al-Amoodi, Asma - *Bioscience, KAUST, Thuwal, Saudi Arabia*
Ait Lahcen, Abdellatif - *Electrical and Computer Engineering, KAUST, Thuwal, Saudi Arabia*
Rauf, Sakandar - *Electrical and Computer Engineering, KAUST, Thuwal, Saudi Arabia*
Salama, Khaled - *Electrical and Computer Engineering, KAUST, Thuwal, Saudi Arabia*
Merzaban, Jasmeen - *Bioscience, KAUST, Thuwal, Saudi Arabia*

Abstract: Exosomes are small extracellular membrane vehicles carrying cargo such as mRNA, micro-RNA, soluble and transmembrane proteins. They have been isolated from a plethora of cell types including stem cells and are believed to mediate communication between cells locally or at a distance. As a means to better understand how exosomes interact with target cells in order to promote functions, we isolated exosomes from CD34+ acute myeloid leukemia (AML) cell line, KG1a, as a model for AML Stem Cells and focused on analyzing their adhesion and migration capacity. A proteomics analysis of the KG1a-derived exosomes indicated the presence of several proteins related to migration and adhesion. Of note, the exosomes contained ligands for E-selectin, an adhesion molecule constitutively expressed on the bone marrow endothelium that is essential for the recruitment of hematopoietic stem cells to the bone marrow. Extensive Western-blot analysis and fluorescence microscopy using recombinant E-selectin confirmed that KG1a-derived exosomes bound to E-selectin. Moreover, MST analysis unveiled a very strong binding between the KG1a-derived exosomes and the E-selectin with an affinity at the pM level. Interestingly, in vivo IVIS imaging of mice injected with DiR-labeled KG1a-derived exosomes revealed their presence in the bone marrow, among other tissues, which was inhibited when mice were pre-treated with blocking antibody to E-selectin. These results were further verified using exosomes derived from CD34+ bone marrow mononuclear cells from healthy and AML patient samples. This work uncovers mechanistic details related to exosome migration and considers that exosomes could use adhesion molecules to actively and autonomously use cell-cell interactions to direct their migration to tissues in order to transport cargo from one cell to another. Understanding the exosomes' full potential and their accuracy in targeting specific cell types can lead to the development of new methodologies of guided and punctual drug delivery systems .

Keywords: AML Stem Cells, Exosomes, Adhesion and Migration

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DECHIPHERING PRELUEKEMIC CLONAL EXPANSION USING INTEGRATED GENETIC BARCODE TRACKING AND SINGLE-CELL TRANSCRITOME ANALYSES

Bramlett, Charles - *Department of Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Clonal expansion occurs when the progeny of a cell excessively increases in number. It plays a crucial role in the early phase of cancer and many hematopoietic disorders such as myeloproliferative disorders, myelodysplastic syndromes, and leukemias. During disease genesis, clonal expansion is often initiated by somatic mutations, which in turn allow the accumulation of additional molecular changes that lead to disease progression. Although mutations of several leukemia associated genes, such as Tet2, have been shown to induce clonal expansion, these mutations are also found in otherwise healthy individuals without any clonal expansion or disease. Little is known about the differences between normal and preleukemic clonal expansion, particularly with respect to the molecular events underlying their differences. Here we tracked clonal expansion using a genetic barcoding technology in Tet2 inducible knockout mice. And we integrated clonal tracking with droplet-based single cell RNA-sequencing (scRNA-seq) to identify genes that are associated with normal and abnormal levels of clonal expansion. We found that Tet2 deletion causes population-level expansion in specific types of myeloid progenitors and blood cells. Their expansions are driven by just a few clones that we have identified as 'over-expanded' clones. By analyzing their transcriptomes, we found that 'over-expanded' Tet2 knockout clones display unique gene expression characteristics compared with other Tet2 knockout clones. These data revealed that Myc pathways are perturbed in 'over-expanded' Tet2 knockout clones. To determine their roles in Tet2 associated clonal expansion, we are currently performing functional assays using CRISPR/Cas9 and shRNA to modulate these genes in conjunction with Tet2 knockout. Our studies revealed molecular signatures that are associated with preleukemic clonal expansion undetectable using conventional bulk or single cell assays.

Funding Source: The research presented is supported by the National Heart, Lung and Blood Institute grant F31HL149278. Additional support came from Leukemia & Lymphoma Society (LLS-1370-20), R35HL150826, R01HL138225, R01HL135292.
Keywords: preleukemic clonal expansion, genetic barcode tracking, Tet2 knockout

TOPIC: IMMUNE SYSTEM

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WISKOTT-ALDRICH SYNDROME PROTEIN FORMS NUCLEAR CONDENSATES AND REGULATES ALTERNATIVE SPLICING

Yuan, Baolei - *Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Jeddah, Saudi Arabia*
Li, Mo - *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*
Ramos-Mandujano, Gerardo - *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*
Wang, Mengge - *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*
Zhou, Xuan - *Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology, Jeddah, Saudi Arabia*

Abstract: The deficiency of WASP causes Wiskott-Aldrich syndrome (WAS) that manifests in microthrombocytopenia, eczema, recurrent infections, autoimmunity and predisposition to malignancy. WASP is known as an actin nucleation factor that initiates actin polymerization to respond to multiple extracellular stimuli. However, its nuclear role that may explain the mechanism of WAS disease remains poorly defined. We generated three isogenic WAS models using patient induced pluripotent stem cells and genome editing. These models recapitulated WAS phenotypes and revealed that WASP deficiency causes an upregulation of numerous RNA splicing factors and widespread altered splicing. The ChIPseq data showed that WASP binds the promoters of most upregulated splicing factors. Loss of WASP binding to splicing factor gene promoters frequently leads to aberrant epigenetic activation. The altered RNA splicing and high TNF secretion in WASP deficient macrophages can be partially rescued by knocking down SRSF2. WASP interacts with dozens of nuclear speckle constituents and constrains SRSF2 mobility. Using an optogenetic system, we showed that WASP forms phase-separated condensates that encompasses SRSF2, nascent RNA and active Pol II. The role of WASP in gene body condensates is corroborated by ChIPseq and RIPseq. Together our data reveal that WASP is a nexus regulator of RNA splicing that controls the transcription of splicing factors epigenetically and the dynamics of the splicing machinery through liquid-liquid phase separation.

Keywords: Wiskott-Aldrich syndrome, Phase-separated condensates, Alternative RNA splicing

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GENERATION OF HUMAN TONSIL EPITHELIAL ORGANOIDS AS AN EX VIVO MODEL FOR SARS-COV-2 INFECTION

Park, Jun-Yeol - *Microbiology, CHA University School of Medicine, Seoul*
Kim, Han Kyung - *Department of Microbiology, CHA University School of Medicine, Seongnam, Korea*
Kim, Hyeryeon - *Otorhinolaryngology-Head and Neck Surgery, Konkuk University School of Medicine, Seoul, Korea*
Lee, Myoung Kyu - *Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology (KRICT, Daejeon, Korea*
Choi, Woo Hee - *Department of Microbiology, CHA University School of Medicine, Seongnam, Korea*
Jang, Yejin - *Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology (KRICT, Daejeon, Korea*
Shin, Jin Soo - *Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea*
Bae, Dong Hyuck - *Microbiology, CHA University School of Medicine, Seongnam, Korea*
Hyun, Seong-In - *Department of Microbiology, CHA University School of Medicine, Seongnam, Korea*

Kim, Kang Hyun - *Department of Biomedical informatics, CHA University School of Medicine, Seongnam, Korea*
Han, Hyun Wook - *Department of Biomedical informatics, CHA University School of Medicine, Seongnam, Korea*
Lim, Byungho - *Data Convergence Drug Research Center, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea*
Choi, Gildon - *Data Convergence Drug Research Center, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea*
Kim, Meehyein - *Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology (KRICT), Daejeon, Korea*
Lim, Young Chang - *Otorhinolaryngology-Head and Neck Surgery, Konkuk University School of Medicine, Seoul, Korea*
Yoo, Jongman - *Department of Microbiology, CHA University School of Medicine, Seongnam, Korea*

Abstract: The palatine tonsils (hereinafter referred to as “tonsils”) serve as a reservoir for viral infections and play roles in the immune system’s first line of defense. The aims of this study were to establish tonsil epithelial cell–derived organoids and examine their feasibility as an ex vivo model for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The tonsil organoids successfully recapitulated the key characteristics of the tonsil epithelium, including cellular composition, histologic properties, and biomarker distribution. Notably, the basal layer cells of the organoids express molecules essential for SARS-CoV-2 entry, such as angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2) and furin, being susceptible to the viral infection. Changes in the gene expression profile in tonsil organoids revealed that 395 genes associated with oncostatin M signaling and lipid metabolism were highly upregulated within 72 h after SARS-CoV-2 infection. Notably, remdesivir suppressed the viral RNA copy number in organoid culture supernatants and intracellular viral protein levels in a dose-dependent manner. Here, we suggest that tonsil epithelial organoids could provide a preclinical and translational research platform for investigating SARS-CoV-2 infectivity and transmissibility or for evaluating antiviral candidates.

Funding Source: HR16C0002, HI18C2458 ,2018R1D1A1A02050030, 2016R1A5A2012284, 2018R1D1A1A02086084, 2021R1A2C2004382 ,20009773 ,NRF-2020M3A9I2108564 ,SI2232-20 NCCP No., 43326
Keywords: tonsil epithelial organoid, SARS-CoV-2, antiviral, transcriptome

TOPIC: KIDNEY

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EX VIVO PERFUSION USING A MATHEMATICAL MODELED, CONTROLLED GAS EXCHANGE SELF-CONTAINED BIOREACTOR CAN MAINTAIN AN ISOLATED MOUSE KIDNEY OR PANCREAS

Rogers, Ian M. - *Research, Mount Sinai Hospital, Toronto, ON, Canada*
Won, Natalie - *Engineering, McMaster University, Hamilton, ON, Canada*
Castillo-Prado, Jorge - *Physiology, University of Toronto, ON, Canada*
Fantin, Amanda - *Physiology, University of Toronto, ON, Canada*
Bhadwal, Anupama - *Physiology, University of Toronto, ON, Canada*
Cho, Alex - *Physiology, University of Toronto, ON, Canada*
Tan, Xinzhu - *Research, McGill University, Montreal, QC, Canada*
Ford, John - *Chemistry, University of Toronto, ON, Canada*
Heath, David - *Chemistry, University of Toronto, ON, Canada*
Mazilescu, Laura - *Surgery, University of Toronto, ON, Canada*
Slezner, Markus - *Surgery, University of Toronto, ON, Canada*

Abstract: An ex vivo organ perfusion system that can maintain isolated organs and optimally resembles the in-vivo environment is ideal since it allows for tightly controlled conditions leading to minimal variation between experiments, no after-surgery care, and reduction of resources required to maintain live animals. Continuous longitudinal observations in real time and without the need for invasive procedures can be done with the ex vivo system. Our Goal is to develop an ex vivo organ culture platform that can be applied to disease modeling and high throughput drug and cell therapy discovery and validation. Our group is developing a 3D tissue/organ culture system that offers the simplicity of cell culture and the depth of animal studies. We can demonstrate that we can maintain an organ for weeks ex vivo and we can replicate disease models using our ex vivo organ that is equivalent to whole mouse disease models. By providing the complexity of whole organs in an easily accessible system our platform is a complement to traditional cell therapy and drug discovery platforms. Importantly, these studies will allow us to develop an ex vivo humanized mouse organ platform by combining decellularized mouse kidneys with human cells therefore providing mini-human organs for pre-clinical tests. We are focusing on the mouse so we can take advantage of the hundreds of available mouse models of human diseases. We have designed a bioreactor that provides sufficient oxygenation through passive diffusion that an external oxygenator or oxygen tank is not required. The bioreactor allows for artery and vein cannula hook ups for two organs for easy oxygen sensor readings in real time. For kidney the ureter can also be cannulated and urine can be sampled in real-time. Using specialized medium we have demonstrated that a mouse kidney can be maintained and remains functional for 9-21 days. We have also demonstrated that we can replicate the unilateral ureteral obstruction model for generating kidney fibrosis in the isolated kidney. Initial work with a mouse pancreas demonstrated the pancreas can be maintained and a responsive to glucose for four days. We are extending these initial findings for both organs.

Funding Source: Canadian Donation and Transplantation Research Program (CDTRP) Banting and Best Diabetes Center Drucker Family Innovation Fund Grant
Keywords: Ex vivo organ perfusion, Mouse kidney disease model, Mouse pancreas disease models

TOPIC: LIVER

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MODELING NON-ALCOHOLIC FATTY LIVER DISEASE IN IPSC-DERIVED HEPATOCYTES

Pastrana, Karina I. - *Biology, San Diego State University /CIRM, San Diego, CA, USA*
Ordonez, Maria - *Department of Pediatrics UC San Diego Health, Sanford Consortium for Regenerative Medicine, San Diego, CA, USA*

Abstract: Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in adults and children worldwide. Despite intensive research, treatment options for NAFLD are lim-

ited, and the disease generates significant financial and public health burdens. Genome-wide association studies discovered a polymorphism in the PNPLA3 gene that confers a higher risk of developing inflammation as a result of NAFLD, and of progressing to liver fibrosis. The mechanism by which the variant allele affects NAFLD progression is poorly understood. Induced pluripotent stem cells (iPSCs) are used as a reliable source of liver cells due to their unlimited expansion and differentiation potential. Our previous studies have shown that iPSC-derived hepatocytes with the PNPLA3 variant allele accumulate more lipid droplets and have increased activation of genes involved in lipid processing and inflammation. Hepatic stellate cells (HSCs) are resident fibroblasts in the liver that play important roles in the healing response, but may also contribute to fibrosis as a result of chronic injury. Understanding the role of the PNPLA3 variant allele in HSCs has a greater potential of preventing liver fibrosis, the most debilitating outcome of NAFLD. We propose to use iPSCs to answer two related questions; 1) does the variant allele enhance the activation state of HSCs, and 2) what are the mechanisms by which fat accumulation in the variant hepatocytes lead to inflammation and HSC activation. For this purpose, we are generating HSCs from iPSC with the wild-type and variant PNPLA3 alleles. We will treat iPSC-HSC with environmental mediators that are known to induce injury in NAFLD, such as palmitic acid, to mimic liver toxicity. We will then measure HSC activation in both wild-type and variant genotypes. We aim to further understand the role that the PNPLA3 variant allele confers on the pathogenesis of NAFLD.

Funding Source: San Diego State University Research Foundation and California Institute for Regenerative Medicine
Keywords: Liver, Disease, Stem Cells

TOPIC: MUSCULOSKELETAL

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MULTIPLE ISOGENIC GNE-MYOPATHY DISEASE MODELING WITH MUTATION SPECIFIC PHENOTYPES FROM HUMAN PLURIPOTNET STEM CELLS BY BASE EDITORS

Park, Ju-Chan - *College of Pharmacy, Seoul National University, Seoul, Korea*
Cha, Hyuk-Jin - *College of Pharmacy, Seoul National University, Seoul, Korea*
Bae, Sangsu - *Institute for Convergence of Basic Sciences, Hanyang University, Seoul, Korea*
Choi, Hyewon - *Department of Life Sciences, Pohang University of Science and Technology, Pohang, Korea*
Jang, Hyun-Ki - *Institute for Convergence of Basic Sciences, Hanyang University, Seoul, Korea*
Kim, Jumee - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kim, Keun-Tae - *College of Pharmacy, Seoul National University, Seoul, Korea*
Kwon, Eun-Ji - *College of Pharmacy, Seoul National University, Seoul, Korea*
Lee, Hyun Sik - *Department of Biological Sciences, Seoul National University, Seoul, Korea*
Lee, Seung-Yeon - *College of Pharmacy, Seoul National University, Seoul, Korea*
Park, Seokwoo - *Department of Biomedical Sciences, Seoul National University, Seoul, Korea*
Park, Seung-yeol - *Department of Biological Sciences, Pohang University of Science and Technology, Pohang, Korea*

Abstract: Despite the great potential of disease modeling using human pluripotent stem cells (hPSCs) derived from patients with mutations, lack of an appropriate isogenic control hinders a precise phenotypic comparison due to the bias arising from the dissimilar genetic backgrounds between the control and diseased hPSCs. Herein, we revealed high expression of uracil DNA glycosylase (UNG) is inhibiting efficiency of cytosine base editor (CBE) and took advantage of currently available base editors (BEs) to epitomize the isogenic disease model from hPSCs. By co-treating siRNA targeting UNG, product purity and efficiency of CBE was significantly increased. Furthermore, we established multiple isogenic GNE myopathy disease models that harbor point mutations on the GNE gene, including four different mutations found in GNE myopathy patients. Four different mutations in the epimerase or kinase domains of GNE revealed mutation-specific hyposialylation, which was closely correlated to pathological clinical phenotypes. GNE protein structure modeling based on the mutations, addressed these mutation-specific hyposialylation patterns. Furthermore, treatment with a drug candidate currently under clinical trials showed a mutation-specific drug response in GNE myopathy disease models. These data suggest that derivation of multiple isogenic disease models from hPSCs by using genome editing can enable translationally relevant studies on the pathophysiology of GNE myopathy and drug responses.

Keywords: Base editor, Human pluripotent stem cells (hPSCs), Disease modeling

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DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TO MESENCHYMAL AND CHONDROCYTIC PHENOTYPES

O'Brien, Aisling - *Regenerative Medicine Institute, National University of Ireland Galway, Ireland*
Barry, Frank - *Regenerative Medicine Institute, National University of Ireland Galway, Ireland*

Abstract: Induced pluripotent stem cells (iPSCs) are considered a potential source of chondrocytes for the treatment of both acute cartilage injury and chronic osteoarthritis. Generating a pure population of hyaline chondrocytes from iPSCs (iCHOs) remains a challenge, and heterogeneity of the differentiated cell population is still common. Current protocols guide iPSCs toward a chondroprogenitor (iCP) or mesenchymal stromal cell (iMSC) intermediate before differentiation to mature iCHOs. Successful approaches have used fluorescent reporter iPSC lines and FACS to obtain purified cell populations expressing chondrocyte or MSC markers. The objectives of this study were to identify a successful and reproducible chondrogenic differentiation method without cell sorting, and to compare the chondrocyte differentiation efficiency of iCPs and iMSCs. Following previously published methods with some modifications, iCPs were generated via the paraxial mesoderm lineage, iMSCs were generated via the neural crest cell lineage, and both cell types were differentiated into iCHOs for 28 days in pellet culture. P0 and P1 iCPs (n=3 individual experiments, N=4 iPSC lines) underwent chondrogenesis in the presence of 10 ng/ml TGFb3 with positive staining for cartilage proteoglycans and produced sulphated glycosaminoglycans (sGAG). Some pellets from P0 iCPs cultured without TGFb3 were capable of proteoglycan synthesis and sGAG production, possibly due to the inclusion of BMP4 in the iCP medium. iMSCs were expanded in 5 ng/ml FGF2 and used for tri-lineage differentiation at P3 (n=3 individual experiments, N=1 iPSC line). iMSCs demonstrated variable population doubling times and a poor capacity to undergo chon-

drogenesis with no sGAG production or proteoglycan synthesis. iMSCs showed high osteogenic and low adipogenic propensity, suggesting that iMSCs produced using current approaches are distinct from primary tissue-derived MSCs. Further strategies being assessed to maintain or enhance differentiation efficiency include the use of additional growth factors and small molecules, and carrying out a screening experiment with iCPs to identify factors that enhance chondrogenesis. In conclusion, differentiating iPSCs toward iCPs for chondrogenesis is a more successful approach that is reproducible across multiple cell lines.

Funding Source: This research is funded by the Celtic Advanced Life Science Innovation Network (CALIN)
Keywords: Chondrogenesis, Induced Pluripotent Stem Cells, Differentiation

TOPIC: NEURAL

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CASC3 SUPPRESSION PROMOTES NEURON SURVIVAL AND MITIGATES TDP-43 PATHOLOGY IN MODELS OF ALS/FTD

Rubin-Sigler, Jasper - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Tu, Sharon - *Dornsife Natural Sciences, University of Southern California, Los Angeles, CA, USA*
Ichida, Justin - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterized by the progressive loss of the upper and lower motor neurons of the brain and spinal cord respectively. ALS affects approximately 30,000 people within the United States and is ultimately fatal, with a life expectancy of 2-5 years following diagnosis. The disease remains incurable and while significant effort has gone into understanding the genetic etiology of ALS, more than 90% of ALS patients have unidentified genetic driver(s) making their pathology challenging to study and cure. However, the nuclear clearance and cytoplasmic aggregation of the RNA binding protein TDP-43 is a hallmark feature observed in 97% of patients regardless of genotype. TDP-43 is a primarily nuclear RNA binding protein that can shuttle between the nucleus and cytoplasm to perform an array of cellular functions and it's mislocalization can induce both loss- and gain-of-function consequences that drive neurodegeneration. To this end, utilizing a 3D-spheroid culture system of induced neurons (iNs) derived from a patient carrying a causal TDP-43 mutation (I383V), we performed an unbiased genome-wide CRISPR-interference (CRISPRi) phenotypic screen to rescue neuron survival. Our results identified a component of the exon junction complex, CASC3, to potently increase neuronal survival in vitro. To validate our screen results, we utilized two CASC3 sgRNAs and a non-targeting sgRNA and found survival to be significantly increased following CASC3 suppression (p=.0269 and p=.0035 for each sgRNA respectively) and to the level of healthy control 3D-iNs. Further, we observed that CASC3 suppression significantly ameliorated the hallmark TDP-43 cytoplasmic mislocalization (p=.0251) and was able to significantly reduce the inclusion of the STMN2 cryptic exon (p=.0037), while increasing the expression of the full-length STMN2 nearly two-fold (p=.013). Our results suggest that CASC3 suppression may help to prevent TDP-43 loss-of-function toxicity but may also mitigate TDP-43 gain-of-function pathology. Together, these data illuminate a potential therapeutic target for a highly conserved element of neurotoxicity across diverse forms of ALS.

Keywords: ALS, Neurodegeneration, TDP-43

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A SCALABLE PLATFORM OF DIFFERENTIATING OLIGODENDROCYTE PROGENITOR CELLS IN 3D SUSPENSION CULTURE

Starikov, Lev - *Neurology, BlueRock Therapeutics, Middle Village, NY, USA*
Cutia, Thomas - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Hovhannisyan, Arno - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Ebel, Mark - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Lepack, Ashley - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Stitt, Nick - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Simpson, Libby - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Adams, Nathaniel - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Michael, Kevin - *Neurology, BlueRock Therapeutics, New York, NY, USA*
LoSchiavo, Deven - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Pereira, Elizabeth - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Srinivas, Maya - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Wilkinson, Dan - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Wang, Jing - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Paladini, Carlos - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Patsch, Christoph - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Irion, Stefan - *Neurology, BlueRock Therapeutics, New York, NY, USA*
Hsu, Juliana - *Neurology, BlueRock Therapeutics, New York, NY, USA*

Abstract: Oligodendrocyte progenitor cells (OPCs) are resident glial cells in the CNS that are highly mobile and readily differentiate into axon-wrapping mature oligodendrocytes throughout lifetime. Emerging cell replacement therapies leverage OPCs in treating demyelinating conditions in which endogenous OPCs are dysfunctional. Derivation of OPCs from induced pluripotent stem cells (iPSCs) provides a promising platform with allogenic capability. However, major obstacles remain in transforming production of oligodendrocyte lineage cells from petri-dish to the clinical scale (estimated >10^8 cells per demyelinated lesion), and a prolonged timeframe compared to most neuronal differentiations. Here we describe a scalable differentiation platform that enables the production of functional OPCs in 60 days in vitro. First phase of dual-SMAD inhibition coupled with activation of sonic hedgehog signaling induced robust co-expression of OLIG2 and NKX2.2 in over 70% of neural progenitor cells (NPCs). These NPCs were then moved to suspension culture to

form oligospheres in either stationary culture or impeller-driven mini-bioreactors for the remainder of the differentiation. We showed that oligospheres generated in bioreactors allowed for a 4-fold expansion in cell yield and improved physical uniformity of spheres. By Day 60, OPCs expressed lineage markers CD9, O4, SOX10, OLIG2, and NKX2.2. Cells generated by stationary culture and bioreactors shared remarkable similarities in protein and gene expression. Notably, bioreactor generated OPCs exhibited strengthened extracellular contacts that led to differential enzymatic selections during dissociation. We found measurement of lactate dehydrogenase release serves a useful cell health indicator during sphere dissociation. Lastly, we showed that the PSC-derived OPCs successfully engrafted and matured into myelin basic protein (MBP) -expressing oligodendrocytes in the hypomyelination mouse brain. Our data support the scalability of a closed system that holds great potential to meet the dose requirement for clinical applications.

Keywords: oligodendrocyte differentiation, bioreactors, glial cell therapy

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A FLUORESCENT TOOLBOX TO DISSECT MITOCHONDRIAL AND LYSOSOMAL MECHANISMS UNDERLYING NEURODEGENERATION

Goldman, Camille - *Black Family Stem Cell Institute, Department of Cell, Developmental & Regenerative Biology, Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Reyes, Ricardo - *Black Family Stem Cell Institute, Department of Cell, Developmental & Regenerative Biology, Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Kareva, Tatyana - *Black Family Stem Cell Institute, Department of Cell, Developmental & Regenerative Biology, Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Ahfeldt, Tim - *Black Family Stem Cell Institute, Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Blanchard, Joel - *Black Family Stem Cell Institute, Department of Cell, Developmental & Regenerative Biology, Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Abstract: Neurodegenerative diseases have innumerable root causes and affect different neuronal sub-types and brain regions but share molecular and cellular pathologies, including mitochondrial and lysosomal dysfunction. Induced pluripotent stem cell (iPSC)-derived models of the human brain are emerging as a powerful tool to study the mechanisms of neurodegeneration. However, we lack probes that enable continuous, real-time monitoring of molecular phenotypes and standardized assays to measure these differences. Quantitative visualization of mitochondrial and lysosomal networks is crucial to better understand how these networks are dysregulated during pathogenesis and lead to eventual degeneration. There are numerous techniques already developed to visualize cellular components, but many have limiting factors; immunofluorescence only offers a snapshot in time and commercial dyes are subject to non-linear uptake caused by slight variations in cell number or culture conditions, making them inconsistent across samples. Genetically encoded fluorescent markers are a powerful tool to consistently visualize lysosomal and mitochondrial networks in live cells without disrupting cellular processes. We have assembled a toolbox of genetically encoded fluorescent markers, introduced by lentivirus, that can be used to interrogate changes in lysosomal and mitochondrial function in cell models of disease pathogenesis and progression. The fluorophores in our toolbox are designed to specifically localize to the desired organelle and illuminate localization, distribution, and number, as well as turnover, recycling by autophagy, and intracellular oxidative stress. The toolbox can be expanded to include existing or new fluorophores and targeted to any subcellular structure or cell-type, greatly expanding the potential for genetically encoded fluorescent markers to probe any cell-model system or subcellular network. We have introduced these genetically encoded fluorescent markers into iPSC-derived neurons and glial cells to visualize mitochondrial and lysosomal dynamics in real-time. We used deep learning to perform semantic segmentation of the organelles to systematically quantify the signals. This is providing new insight into the neurodegenerative mechanisms underlying Parkinson's disease.

Keywords: Genetically encoded fluorescent markers and live-cell microscopy, Real-time lysosomal and mitochondrial dynamics, Deep learning signal analysis

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THE EFFECTS OF PRENATAL EXPOSURE OF GABAPENTINOIDS ON HUMAN CORTICAL NEURONS

Alsanie, Walaa - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Althobaiti, Yusuf - *College of Pharmacy, Taif University, Taif, Saudi Arabia*
Alamri, Abdulhakeem - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Alhomrani, Majid - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Aljuaid, Wasaif - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Almalki, Eman - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Aloisami, Ebtisam - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Alshehri, Amjad - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Alsofuani, Nada - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Althobaiti, Nojoud - *Clinical Laboratories Sciences, Taif University, Taif, Saudi Arabia*
Bahri, Ola - *Biology Department, Taif University, Taif, Saudi Arabia*
Gaber, Ahmed - *Biology Department, Taif University, Taif, Saudi Arabia*
Habeeballah, Hamza - *Clinical Laboratories Sciences, KAAU University, Jeddah, Saudi Arabia*
Haynes, John - *Monash University, Monash University, Melbourne, Australia*
Pouton, Colin - *Monash University, Monash University, Melbourne, Australia*

Abstract: Prenatal substance exposure is a major public health concern that is associated with many detrimental fetal consequences. Unfortunately, polysubstance use in pregnancy is common. Gabapentinoids are widely used as treatments in psychiatry and neurology; however, they are increasingly being reported as having potential for misuse. Moreover, it is already known that gabapentinoids are able to cross the placental barrier. Due to difficulties in accessing fetal brains exposed to gabapentinoids, we used the human embryonic stem cell (hESC) line H9 to generate early, intermediate cortical progenitors and cortical neurons, to modulate the prenatal exposure of gabapentinoids in vitro. Since cortex is responsible for cognition and behavior, we focused on cortical development. We have analyzed the treated (10uM) and untreated (control) cultures for gene expressions, neurogenesis and morphogenesis. At the early patterning stage, there was a significant increase in Tbr2+ intermediate progenitors in pregabalin- and gabapentin-treated cultures. In addition, there was a significant increase in the expression in cortical related genes Pax6, Foxg1 and Tbr2 in pregabalin treated cultures, whereas gabapentin significantly increased Tbr2 expression solely. At the maturation stage, the number of cortical mature neurons was not changed in pregabalin treated cultures. However, gabapentin significantly increased Tbr1+ neurons but not Ctip2+ neurons at early maturation. At the genetic level, we screened the effects of pregabalin on different cortical layers related genes. Pregabalin has significantly increased the expression of Brn2 with no significant effect on the other screened genes. On the other hand, gabapentin did not alter any cortical layer related genes. In term of morphogenical analysis, both pregabalin and gabapentin significantly decreased the neurites length, branches and neurites of human cortical neurons. Our data also show that the effects of pregabalin and gabapentin on the morphogenesis of cortical neurons differ based on the presence of maturation factors, such as GDNF and BDNF. Our study demonstrates that the exposure to gabapentinoids during early brain development could interfere with the neurogenesis and morphogenesis of different neuronal subpopulations.

Funding Source: the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this work through project number 1-441-133.
Keywords: Neural Development, Drug Testing, Disease Modeling

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SPINAL CORD CHIP FROM SPORADIC ALS PATIENTS REVEAL DISEASE SPECIFIC BIOMARKERS

Lall, Deepti - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ondatje, Briana - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Bell, Shaughn - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Sances, Samuel - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Workman, Michael - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Van Eyk, Jennifer - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Svendsen, Clive - *Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a complex neurodegenerative disorder, with the vast majority of cases being sporadic in nature with unknown genetic cause. ALS is characterized by progressive loss of cortical and spinal cord motor neurons, resulting in muscle weakness and death from respiratory failure typically within 3-5 years of diagnosis. Using induced pluripotent stem cell (iPSC) technology, patient-specific cells can be differentiated into cell types relevant to ALS. Microphysiological systems (MPS), also known as organ-chips, have microengineered three dimensional compartments that enable the co-culture of different iPSC-derived cell types to recapitulate conditions of human physiology in vitro. Here we developed an MPS-based model incorporating patient-derived spinal cord motor neurons (SC-Chip) to study young onset sporadic ALS. The SC-Chip top channel contains disease-relevant neuronal cells, and the bottom channel contains brain microvascular endothelial cells (BMECs). The porous membrane between the two channels allows for the establishment of a blood-brain barrier. The primary goal of this project is to establish robust and reproducible biomarkers of this disease by identifying metabolomic, transcriptomic, and proteomic signatures, as well as to test efficacy of candidate therapeutics to ameliorate disease-specific phenotypes. We have detected several biomarkers of sporadic ALS that are consistent across multiple studies in RNA-seq, proteomics, immunohistochemical staining and western blot analysis. Identified biomarkers for sporadic ALS include increased expression of heavy, medium, and light chain neurofilaments as well as peripherin, an increase in calmodulin binding substrate neurogranin, and a decrease in neural RNA-binding protein ELAV3. We will next administer experimental therapeutics to the vascular side of the SC-Chip to determine (i) transport across the blood-brain barrier to identify novel biomarker modulating compounds and (ii) reversal of disease-specific phenotypes on the neuronal side upon drug treatment.

Funding Source: This study is funded by NIH grant 5UG3NS105703-02
Keywords: Microphysiological Organ-on-Chip System, Amyotrophic Lateral Sclerosis, Biomarkers

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SCALABLE DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO NEURONAL LINEAGE CELLS USING BENCHTOP BIOREACTORS

Wihan, Jeanette - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany*
Karnatz, Isabell - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Würzburg, Germany*
Sébastien, Isabelle - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Würzburg, Germany*
Fischer, Benjamin - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Würzburg, Germany*
Schmid, Benjamin - *Bioneer A/S, Hoersholm, Denmark*
Clausen, Christian - *Bioneer A/S, Hoersholm, Denmark*
Cabrera-Socorro, Alfredo - *Neuroscience Therapeutic Area, Janssen Research & Development, Beerse, Belgium*
Steeg, Rachel - *Technology and Innovation Centre, Fraunhofer UK Research Ltd, Glasgow, UK*
Neubauer, Julia - *Fraunhofer Project Center for Stem Cell Process*

Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Würzburg, Germany
Zimmermann, Heiko - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Würzburg, Germany*

Abstract: Human induced pluripotent stem cells (hiPSCs) represent a valuable and versatile tool for research and drug development. To meet the increasing demand in high-quality hiPSCs, the European Bank for induced pluripotent Stem Cells (EBiSC) was founded in 2014. By integrating state-of-the-art upscaling and automation, EBiSC provides a central platform for standardized cells and operating procedures. Given the global socioeconomic burden of neurological diseases, one major focus of its second project phase (EBiSC2) has been drawn on differentiated products from well-characterized hiPSCs, especially neuronal lineage cells. We present a fast and efficient protocol for the scalable differentiation of high quality hiPSCs into neurons using a benchtop 3D cell culture incubator. After transferring adherent hiPSC cultures into suspension, aggregates were formed on a short term, and neural lineage commitment was induced by the ectopic expression of the neural transcription factor neurogenin 2. The generated neurons expressed classical neuronal markers early on and formed complex neuritic networks within one week after replating. In a second approach, we have used alginate microcarriers as scaffolds for neuronal differentiation. The cells were seeded as monolayer on matrigel-coated carriers. We assessed yield potential and neuronal gene expression similar to our standardized aggregate culture. As cells were observed to detach easily from matrigel-coated carriers, we tested different surface coatings to improve cell adhesion and neurite formation. In summary, we demonstrate the scalable generation of neurons in a 3D environment, which serves as a starting point for high throughput drug screenings and large-scale toxicity assays.

Funding Source: This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 821362. The JU receives support from the European Union's Horizon 2020 RIA programme and EFPIA.
Keywords: NGN2 induced neurons, EBiSC2, Benchtop Bioreactors

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PROTEOMICS ANALYSIS OF ALEXANDER DISEASE PATIENT IPSC-DERIVED ASTROCYTES IDENTIFIES NEW PATHOPHYSIOLOGICAL FEATURES OF THE DISEASE AND DRUGGABLE TARGETS

Jovanovic, Vukasin M. - *Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), Rockville, MD, USA*
Braisted, John - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Sen, Chaitali - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Tristan, Carlos - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Ormanoglu, Pinar - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*
Singeç, Ilyas - *Division of Preclinical Innovation, NCATS, Rockville, MD, USA*

Abstract: Alexander disease (AxD) is a rare and fatal leukodystrophy caused by mutations in the GFAP gene resulting in protein aggregates known as Rosenthal fibers (RF). We recently demonstrated that astrocytes derived from an AxD patient recapitulate molecular hallmarks of disease including RF formation, activation of stress pathways, and elevated levels of autophagy. Here, we performed two-dimensional gel electrophoresis of membrane-bound fraction of proteins isolated from healthy and diseased iPSC-astrocytes under basal conditions and after 24 h treatment with inflammatory cytokines. This approach enabled detection of differentially expressed proteins under basal conditions and after cytokine stimulation. A total of 92 hits were detected and all proteins were identified by MALDI/TOF mass spectrometry. Top upregulated hits in AxD astrocytes included ER chaperons involved in protein folding (CALR, CALU), cytoskeletal proteins (VIM, ACTG, TPM1/3), cell adhesion molecules (VCAM1, ITA2), oxidative stress (SODM), and innate immunity (C1QBP) related proteins. Among top downregulated hits were proteins and enzymes regulating metabolic pathways and energy such as glycolysis (ENOA, GAPDH), pyruvate cycle (MAOM), antioxidant activity (PRDX2) and NADH redox balance (AATM). Exposure to inflammatory cytokines had a particularly strong effect and decreased the levels of glycolytic enzymes (GAPDH, ENOA) in AxD astrocytes. These findings were functionally validated by real-time metabolic analysis using a standardized glycolysis stress test (Searhorse XF Analyzer). Next, guided by the identified hits, we queried the NIH Pharos database (https://pharos.nih.gov) to find potential disease-modifying drugs. Indeed, 18 out of 92 proteins could be linked to small molecule drugs that may modulate disease-relevant processes and are currently being studied in more detail.

Keywords: Alexander disease, iPSC-Astrocytes, Drug target identification

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PATIENT DERIVED IPSC MODEL FROM TRANSANCESTRY SAMPLES IMPLICATES SYNAPTIC FUNCTION IN SCHIZOPHRENIA

Tumkaya, Tayfun - *NIBR, Novartis, Cambridge, MA, USA*
Bilican, Bilada - *NIBR, Novartis, Cambridge, MA, USA*
Chan, Karrie - *NIBR, Novartis, Cambridge, MA, USA*
Chen, Julie - *NIBR, Novartis, Cambridge, MA, USA*
Fletcher, David - *NIBR, Novartis, Cambridge, MA, USA*
Henry, Beata - *NIBR, Novartis, Cambridge, MA, USA*
Kaykas, Ajamete - *NIBR, Novartis, Cambridge, MA, USA*
Kommineni, Sravya - *NIBR, Novartis, Cambridge, MA, USA*
Mansur, Leandra - *NIBR, Novartis, Cambridge, MA, USA*
Mull, Jesse - *NIBR, Novartis, Cambridge, MA, USA*
Ruda, Vera - *NIBR, Novartis, Cambridge, MA, USA*
Sondey, Marie - *NIBR, Novartis, Cambridge, MA, USA*
Theriault, Kraig - *NIBR, Novartis, Cambridge, MA, USA*
Wang, Yanqun - *NIBR, Novartis, Cambridge, MA, USA*
Worringer, Kathleen - *NIBR, Novartis, Cambridge, MA, USA*
Dolmetsch, Ricardo - *NIBR, Novartis, Cambridge, MA, USA*
Chung, Jonathan - *NIBR, NOVARTIS, Cambridge, MA, USA*
Ihry, Robert - *NIBR, NOVARTIS, Cambridge, MA, USA*

Abstract: Genetic predisposition is a major risk factor for schizophrenia (SZ). While genome–wide association studies (GWASs) with large sample sizes have linked hundreds of genetic loci to SZ, functional genomics studies are critical for mapping out the downstream targets of these loci. We developed an automated platform to culture induced pluripotent stem cells (iPSCs) from 40 childhood-onset schizophrenia (COS) cases and controls with mainly European (EUR) and African (AFR) genetic ancestries. Our

transcriptomic analyses of the bulk RNA–seq data collected from the patients revealed a dysregulation in the expression of SZ–related ion–channel and synaptic genes. An association analysis between genetic variants and gene expression levels (i.e., cis–eQTL analysis) discovered 1,097 putative genes that could mediate the interaction between the genetic loci and SZ. Moreover, 94 of the cis–eQTL variants that regulate the gene expression also colocalize with SZ genetic risk loci identified by the GWAS studies, prioritizing novel target genes. We show that while transcriptomic signature of SZ is heterogenous across patients with diverse genetic backgrounds, carefully designed functional genomics studies can help elucidate the disease biology.

Keywords: iPSC, Schizophrenia, Polygenic risk score (PRS)

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PAR1 CONTRIBUTION TO NEURODEVELOPMENTAL DEFECTS IN PATIENTS WITH KLINEFELTER SYNDROME AND HIGH-GRADE X CHROMOSOME ANEUPLOIDIES

Adamo, Antonio - *Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Astro, Veronica - *Biological and Environmental Sciences and Engineering Division, KAUST University, Thuwal, Saudi Arabia*
Colombo, Giovanni - *Biological and Environmental Sciences and Engineering Division, KAUST University, Thuwal, Saudi Arabia*
Cardona-Londoño, Kelly - *Biological and Environmental Sciences and Engineering Division, KAUST University, Thuwal, Saudi Arabia*
Fiacco, Elisabetta - *Biological and Environmental Sciences and Engineering Division, KAUST University, Thuwal, Saudi Arabia*
Saera-Vila, Alfonso - *Sequentia Biotech, Sequentia Biotech SL, Barcelona, Spain*

Abstract: Klinefelter syndrome (KS) is the most prevalent aneuploidy in males and is characterized by a 47, XXY karyotype. Less frequently, higher grade sex chromosome aneuploidies (HGAs) can also occur. KS and HGA patients present a broad spectrum of clinical manifestations, including infertility, intellectual disability, cardiac abnormalities, metabolic disorders, and cancers. We previously generated a paradigmatic cohort of KS and HGA induced pluripotent stem cells (iPSCs) to investigate the transcriptional consequences of X chromosome overdosage in 49, XXXXY, 48, XXXY, and 47, XXY patients. We identified genes within the pseudoautosomal region 1 (PAR1) as the most susceptible to dosage-dependent transcriptional dysregulation. Moreover, we proved the transcriptional impact of X overdosage on autosomes and demonstrated that the autosomal transcription factor NRF1 is a direct regulator of the X-linked gene ZFX. However, it remains unclear which genes are responsible for the symptoms associated with KS patients and which is the mechanism leading to the progressively worsening clinical conditions, cognitive deficit, language impairment, and autistic spectrum disorders observed in HGA. We generated male H1 hESCs overexpressing a subset of PAR1 genes to address these questions. We then differentiated hESCs overexpressing single PAR1 genes, KS-iPSC and HGA-iPSCs into neural stem cells (NSCs) and neurons for dissecting PAR1 contribution to KS and HGA neuropathophysiology. This strategy allowed us to profile the transcriptional signature of the neurological impairments caused by PAR1 overdosage and recapitulate the cell state transition during early KS and HGA patients' neurodevelopment. Thanks to our innovative approach, we have identified the PAR1 genes that may contribute to the neurological deficits and cognitive traits of KS and HGA patients and are therefore responsible for the progressively worsening phenotype in higher grade X aneuploidies.

Funding Source: KAUST Baseline fund BAS/1/1077-01-01 to Antonio Adamo
Keywords: Klinefelter Syndrome, Pluripotent Stem Cells, Differentiation

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MONITORING, ANALYSIS AND FUNCTIONAL READOUTS OF IPSC-DERIVED 3D CEREBRAL ORGANOIDS

Lim, Angeline - *Imaging, Molecular Devices, LLC, San Jose, CA, USA*
Sirenko, Oksana - *Imaging, Molecular Devices, LLC, San Jose, CA, USA*
Spira, Felix - *Imaging, Molecular Devices, Salzburg, Austria*

Abstract: Cerebral 3D organoids are a rapidly developing technology that has great potential for understanding brain development and neuronal diseases, and can be used for testing effects of compounds and genetic mutations. There has been great progress in developing methods for culturing brain organoids from induced pluripotent stem cells, that allows characterization of later events in cortical development. Further method development needs to include adoption of the model at scale for testing the effects of compounds and drug candidates, and also development of methods for testing the functional neuronal activity. We describe a method for automated culture and monitoring of cerebral organoids, as well as testing functional neuronal activity, by recording and analyzing Ca2+ oscillations. Cerebral organoids were developed from iPSC using Lancaster and Knoblich 2014 method and Cerebral Organoid Kit (STEMCELL Technologies). We monitored size and morphology of developing brain microtissues in transmitted light over 20 weeks of development, using AI-based image analysis (IN Carta) for defining the size, shape, and density of the tissues. Selected microtissues were analyzed during the different phases of development using confocal imaging by expression of Sox2, TuJ1, and GFAP markers. For detection of functional activities, calcium oscillations from selected clusters of the microtissues were recorded by imaging and analyzed using MetaXpress image analysis software. Oscillations were observed after day 50 in culture and were sporadic through the volume of micro-tissue, while significantly activated by 4-aminopyridine. Cellular viabilities and organoid morphologies were analyzed using viability markers and 3D image analysis. For assay characterization, we used several neuromodulators including 4-aminopyridine, GABA, NMDA, muscimol, etc. Also we tested known neurotoxins rotenone and mercury. The observed changes in oscillation patterns were consistent with expected actions of respective compounds, while degree of baseline activities and drug responses varied for different micro-tissues. The method demonstrates a promise for evaluation of effects of pharmaceutical drugs, toxins, and genetic mutations, while further method development is needed for screening applications.

Keywords: cerebral organoids, monitoring, calcium imaging

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MODELLING PELIZAEUS-MERZBACHER DISEASE IN BIOENGINEERED NEURONAL ORGANOIDS

Schreiber, Marie-Kristin - *Institute for Pharmacology and Toxicology, University Medical Centre Goettingen (UMG), Göttingen, Germany*
Ivics, Zoltán - *Medical Biotechnology, Paul-Ehrlich-Institute, Langen, Germany*
Sebe, Atilla - *Medical Biotechnology, Paul-Ehrlich-Institute, Langen, Germany*
Zafeiriou, Maria-Patapia - *Institute for Pharmacology and Toxicology, University Medical Centre Göttingen, Göttingen, Germany*

Abstract: Pelizaeus-Merzbacher disease (PMD) is an x-linked, hypomyelinating leukodystrophy with no effective treatment. PMD patients present with developmental delays and premature mortality. Genetic alterations in myelin proteolipid protein gene (PLP1), encoding for a major myelin protein, cause impaired myelin sheath formation and induce axonal degeneration in PMD patients. In this study, we hypothesized that we can model and study PMD in our recently developed brain organoid model. Bio-engineered neuronal organoids (BENOs) display self-organisation and neuron and glia co-development with axonal myelination from day 90 of differentiation. We investigated BENOs deriving from induced pluripotent stem cells (iPSCs) of two PMD patients with known disease-causing genetic alterations. Patient A carrying a point mutation (c. 98G-A) and Patient B, a duplication in PLP1 gene (Xq22.3) were compared to a well-characterised control iPSC line (TC1133, Lonza). By day 120, both PMD BENOs (Patient A and B, n=12 BENO / patient, 3 independent experiments) demonstrated lower transcript expression of oligodendrocyte marker Olig2 (A: 314 fold, B: 217 fold), and myelin proteins CNP (A: 1.8 fold, B: 5.5 fold), PLP1 (A: 57 fold, B: 53 fold) and MBP (A: 314 fold, B: 217 fold) compared to control BENOs. Whole mount immunofluorescence (Patient A and B, n=9 BENO / patient, 3 independent experiments) showed that PMD BENOs indeed contained fewer Olig2-positive oligodendrocytes, decreased neuronal innervation, lower levels of axonal myelination, and signs of neuronal damage. Moreover, to validate that c.98G-A is the causing mutation, we CRISPR-engineered a c.98G-A mutant in TC1133. Preliminary morphological and transcriptional profiling of c.98G-A-TC1133 BENOs displayed a similar phenotype to Patient A BENOs, indicating c.98G-A as the causative mutation. In the future, we plan to investigate the underlying mechanism responsible for the observed phenotype and examine differences in neuronal activity using multi electrode assay (MEA) analysis. In conclusion, our data indicate BENOs as a promising preclinical model for gene therapy or genome engineering approaches to treat demyelinating diseases such as PMD.

Keywords: Demyelinating diseases, Human neuronal organoids, Pelizaeus-Merzbacher Disease

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MAKING FUNCTIONAL NEUROMUSCULAR JUNCTIONS FROM HUMAN PLURIPOTENT STEM CELLS THROUGH A NEUROMESODERMAL PROGENITOR INTERMEDIATE STATE

Urzi, Alessia - *House 27, Max Delbruck Center for Molecular Medicine, Germany*
Garcia-Perez, Angelica - *Haus 27, MDC, Berlin, Germany*
Martins, Jorge - *Haus 27, MDC, Berlin, Germany*
Lelievre, Noemie - *Haus 27, MDC, Berlin, Germany*
Bittner, Marie-Sophie - *Haus 27, MDC, Berlin, Germany*
Gouti, Mina - *Haus 27, MDC, Berlin, Germany*

Abstract: In the neuromuscular system, motor neurons and skeletal muscle cells interact together to form highly specialised synapses – called neuromuscular junctions. Together, they control the movements of the body and when their function is compromised it results in neuromuscular disorders. The study and correction of such defects rely on the establishment of accurate human models in vitro. Here, we have successfully used neuromesodermal progenitors – the building blocks of the neuromuscular system – derived from human pluripotent stem cells, to generate a novel 2D neuromuscular junction model. In this in vitro human model, trunk skeletal muscle fibers, spinal cord neurons and terminal Schwann cells are generated simultaneously to form functional networks. Neuromuscular junctions are reproducibly formed and mature over time resulting in the contraction of the skeletal muscle resembling the in vivo situation. Functional connectivity between motor neuron endplates and skeletal muscles is proved by optogenetic analysis and rapid muscle relaxation after treatment with curare, an acetylcholine receptor inhibitor. Finally, we used this neuromuscular junction model to recapitulate key features of spinal muscular atrophy in vitro. This opens the exciting opportunity to study the selective vulnerability of motor neurons and skeletal muscles to neuromuscular diseases.

Keywords: Neuromesodermal progenitors, Neuromuscular junctions, Spinal muscular atrophy

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INVESTIGATION OF GENETIC SEX DIFFERENCES IN HUMAN EMBRYONIC STEM CELLS DURING NEURAL DIFFERENTIATION

Pottmeier, Philipp - *Organismal Biology - Evolution and Development, Uppsala University, Uppsala, Sweden*
Peuckert, Christiane - *Department of Molecular Biosciences, Stockholm University, Stockholm, Sweden*
Jazin, Elena - *Organismal Biology - Evolution and Development, Uppsala University, Uppsala, Sweden*

Abstract: The prevalence, age of onset, and clinical symptoms of many neurological disorders differ substantially between males and females. We hypothesize, that sexual differentiation of the human brain during early development is a major factor contributing to the sex-biased susceptibility of neurological disorders. Relatively little research is being conducted that focuses specifically on genetic sex differences in neurodevelopment. However, there is evidence for sex differences in for example neurogenesis from studies in rodents. To identify the genetic contributors to potential sex differences in early human nervous system development, we have performed a neural differentiation with multiple male and female human embryonic stem cell lines and subsequent-

ly analyzed bulk RNA samples from different time points of the differentiation using RNAseq. The resulting expression profiles revealed a number of sex-biased differentially expressed genes that are implicated in neural development and signaling. After 37 days of differentiation, several genes specifically involved in synaptic signaling and multiple neural transcription factors were upregulated in the male cell lines. Interestingly, we also found sex-biased gene expression as early as 4 days after the start of differentiation. Here, the male cells showed upregulated gene sets involved in actin filament organization, cell-cell and cell-substrate adhesion, whereas the female cell lines showed an increase in gene sets regulating cilium development, movement and cilium-based transport. While these processes are not directly associated with neural differentiation, they can still contribute to a different differentiation of the cell lines. Taken together our results point towards intrinsic differences in differentiation potentials of male and female cell lines.

Funding Source: This study was funded by the Swedish Research Council (project number 2015-03542) and the Foundation for Zoological Research in Uppsala.
Keywords: sex differences, neural differentiation, human embryonic stem cells hESC

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INTEGRATING AUTOMATED HIGH-CONTENT SCREENING WITH DEEP LEARNING TO UNLOCK NOVEL DISEASE SIGNATURES AND CANDIDATE THERAPEUTICS

Paull, Daniel - *Array, The New York Stem Cell Foundation Research Institute, New York, NY, USA*
Schiff, Lauren - *Google Research, Google, Mountain View, CA, USA*
Migliori, Bianca - *Array, NYSCF, New York, NY, USA*
Duren-Lubanski, Alyssa - *Array, NYSCF, New York, NY, USA*
Rusielewicz, Tom - *Array, NYSCF, New York, NY, USA*
Chen, Ye - *Google Research, Google, Mountain View, CA, USA*
Carter, Deidre - *Array, NYSCF, New York, NY, USA*
Bonilla, Caitlyn - *Google Research, Google, Mountain View, CA, USA*
Fischbacher, Brodie - *Array, NYSCF, New York, NY, USA*
Hunter, Christopher - *Array, NYSCF, New York, NY, USA*
Williams, Brian - *Google Research, Google, Mountain View, CA, USA*
Otto, Reid - *Array, NYSCF, New York, NY, USA*
Buckley-Herd, Geoff - *Array, NYSCF, New York, NY, USA*
Horn, Grayson - *Array, NYSCF, New York, NY, USA*
Hazard, Jon - *Google Research, Google, Mountain View, CA, USA*
Goldberg, Jordan - *Array, NYSCF, New York, NY, USA*
Coram, Marc - *Google Research, Google, Mountain View, CA, USA*
Jess, Phillip - *Google Research, Google, Mountain View, CA, USA*
Pritchard, Orion - *Google Research, Google, Mountain View, CA, USA*
Bauer, Lauren - *Array, NYSCF, New York, NY, USA*
Noggle, Scott - *Array, NYSCF, New York, NY, USA*
Monsma, Frederick - *Array, NYSCF, New York, NY, USA*
Berndl, Marc - *Google Research, Google, Mountain View, CA, USA*
Yang, Samuel - *Google Research, Google, Mountain View, CA, USA*
Johannesson, Bjarki - *Array, NYSCF, New York, NY, USA*

Abstract: Progress in drug discovery and early diagnostics for complex diseases has been limited by the lack of screenable phenotypes in scalable cell models. Here we present a novel unbiased phenotypic profiling platform that combines high-throughput cell culture automation, Cell Painting, and deep learning. We will present data from work performed across two different diseases: Parkinson's Disease and the rare pediatric disorder, Infantile Neuroaxonal Dystrophy. In both cases, we applied our platform to primary fibroblasts from large cohorts of patients and carefully matched controls. Using fixed weights from a convolutional deep neural network trained on ImageNet, we generated unbiased deep embeddings from each image and applied these to train machine learning models to detect morphological disease phenotypes. Our models captured individual variation by identifying specific cell lines within the cohort with high fidelity, even across different batches and plate layouts, demonstrating platform robustness and sensitivity. Our models are able to confidently separate healthy controls from both Parkinson's patients and INAD patients. Building upon this work, we will present our latest efforts in applying high-throughput screening to identify novel candidate therapeutics for modulating these diseases. This phenotypic profiling platform, as presented here, can be readily adapted to multiple complex diseases as well as to diverse, disease-relevant cell types including iPSC derivatives. Combined with the large cell line repository that we have here at NYSCF, it holds great potential to uncover morphological signatures of different diseases and conditions and advance precision drug discovery.

Funding Source: NYSCF, Google
Keywords: Parkinson's, Machine Learning, Automation

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INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL ORGANOIDS INCORPORATING MICROGLIA FOR INTERROGATION OF NEURAL INFLAMMATION

Richards, William - *Stem Pharm, Inc., Madison, WI, USA*
Zimmermann, Joshua - *Stem Pharm, Inc., Madison, WI, USA*
Favreau, Peter - *Stem Pharm, Inc., Madison, WI, USA*
Greuel, Kaylie - *Stem Pharm, Inc., Madison, WI, USA*
Parham, Kailyn - *Stem Pharm, Inc., Madison, WI, USA*
Lebakken, Connie - *Stem Pharm, Inc., Madison, WI, USA*

Abstract: Neuroinflammation is a complex response to brain injury involving activation of the innate immune response, release of inflammatory mediators and the generation of reactive species resulting in downstream effects including vascular compromise, oxidative stress, and neurotoxicity. Neuroinflammation is an important component in many disease etiologies including neurodegenerative disease, stroke, trauma, seizures, neuropsychiatric disorders, and brain tumors. There is a critical need to develop advanced neural microphysiological systems (MPS) that can model neuroinflammation. Stem Pharm has leveraged its proprietary synthetic hydrogel platform to enable the formation of complex, reproducible, induced pluripotent stem cell (iPSC)-derived neural organoids containing microglia and vascular cells that are well-suited to study neural inflammation. The neural organoids are formed in 96-well plates from iPSC-derived neural precursor cells, microglia, endothelial cells, and mesenchymal stem cells and ready for screening 21 days after initial plating. Single cell transcriptional analysis demonstrates that the organoids are cell-type diverse, containing multiple neuronal subtypes, astrocytes, microglia, and endothelial cells. Bulk and single cell RNA-seq

analysis demonstrates high intraclass correlation and low coefficients of variation between biological replicates. Microglia incorporated into the organoids are distributed throughout the organoids, display ramified morphology resembling in vivo morphology, and demonstrate a gene signature that strongly correlates with in vivo microglia expression. Modulation of microglia within the organoids to pro- and anti-inflammatory phenotypes was validated through stimulation with lipopolysaccharides, interferon gamma, TGFβ & IL-10, or IL-4 & IL-13. These data demonstrate the promising application of Stem Pharm's advanced neural organoids for facilitating translation between pre-clinical and clinical discovery and development in neuroinflammation.

Funding Source: NIH NIEHS SBIR grants 1R43ES029898-01A1, 1R43ES029897-01, 2R44ES029897-01, and 1R43ES029897-01S1
Keywords: Organoid, Microglia, neuroinflammation

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HUMAN IPSC-DERIVED NEURONS REVEAL EARLY DEVELOPMENTAL ALTERATION OF NEURITE OUTGROWTH IN THE LATE-OCCURRING NEURODEGENERATIVE WOLFRAM SYNDROME

Sciauvaud, Axel - *The Institute for Stem Cell Therapy and Exploration of Monogenic diseases (I-Stem), Paris-Saclay University, Corbeil-Essonnes, France*
Pourtoy-Brasselet, Sandra - *WOLFRAM SYNDROME, I-STEM, Corbeil-Essonnes, France*
Boza-Moran, Maria-Gabriela - *Wolfram Syndrome, I-STEM, Evry, France*
Cailleret, Michel - *Wolfram Syndrome, I-STEM, Evry, France*
Jarrige, Margot - *NGS PLATFORM, I-STEM, Evry, France*
Polveche, Helene - *NGS PLATFORM, I-STEM, Evry, France*
Polentes, Jerome - *HTS PLATFORM, I-STEM, Corbeil-Essonnes, France*
Chevet, ERIC - *"Chemistry, Oncogenesis, Stress, Signaling" (COSS), Universite de Rennes-1 Inserm U1242, Rennes, France*
Martinat, Cecile - *MOTONEURONS DISEASE, I-STEM, Evry, France*
Peschanski, Marc - *I-STEM, Evry, France*
Aubry, Laetitia - *Wolfram Syndrome, I-STEM, Evry, France*

Abstract: Wolfram syndrome (WS), also known as DIDMOAD, is a rare genetic disease characterized by a combination of several symptoms: diabetes insipidus (DI), diabetes mellitus (DM), optic atrophy (OA) and deafness (D). In addition, neurological damage during adolescence is responsible for the premature death of patients. Recent studies indicate that neurodegenerative processes that appear during childhood and adolescence in individuals with WS occur in addition to early brain development alteration, which is clinically silent. Underlying pathological mechanisms are still unknown. We have used induced pluripotent stem cell-derived neural cells from individuals affected by WS in order to reveal their phenotypic and molecular correlates. We have observed that a subpopulation of Wolfram neurons displayed aberrant neurite outgrowth associated with altered expression of axon guidance genes. Selective inhibition of the ATF6α arm of the unfolded protein response prevented the altered phenotype, although acute endoplasmic reticulum stress response-which is activated in late Wolfram degenerative processes-was not detected. Among the drugs currently tried in individuals with WS, valproic acid was the one that prevented the pathological phenotypes. These results suggest that early defects in axon guidance may contribute to the loss of neurons in individuals with WS.

Keywords: Wolfram syndrome, axon guidance and neurite outgrowth defect, neurodevelopmental disease

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DEFINING THE ROLE OF MICROGLIA-RELATED GENETIC RISK VARIANTS IN LATE-ONSET ALZHEIMER'S DISEASE USING HUMAN IPSC MODELS

Rao, Chandrika - *Brain Modeling, The New York Stem Cell Foundation, New York, NY, USA*
Kalpana, Kriti - *Neurodegenerative Diseases, New York Stem Cell Foundation Research Institute, New York, NY, USA*
Kruglikov, Ilya - *Neurodegenerative Diseases, New York Stem Cell Foundation Research Institute, New York, NY, USA*
Gandy, Samuel - *Icahn School of Medicine, Mount Sinai, New York, NY, USA*
Noggle, Scott - *Neurodegenerative Diseases, New York Stem Cell Foundation Research Institute, New York, NY, USA*
Fossati, Valentina - *New York Stem Cell Foundation Research Institute, Neurodegenerative Diseases, New York, NY, USA*

Abstract: Microglia, the resident immune cells of the brain, have now emerged as central players in neuroinflammation and neurodegeneration. Large-scale genetic and computational analyses of human Alzheimer's disease (AD) brains have identified that more than two-thirds of genes implicated in the progression of late-onset AD are expressed either exclusively, or at very high levels, in microglia. The precise functional role of microglia in AD and their interaction with other cell types in the brain, however, remain poorly understood. TREM2 and TYROBP/DAP12, which together form a key microglial signaling complex, have been strongly implicated in the progression of microglia from a homeostatic state to a disease-associated microglial (DAM) phenotype during neurodegeneration. To better understand the role of this signaling pathway in AD we have generated an isogenic panel of CRISPR/Cas9-edited human iPSC lines harboring mutations in TREM2 and TYROBP/DAP12 in the context of clinical and pathology confirmed late-onset AD, and we have differentiated these cells to iPSC-derived microglia. Using a combination of transcriptomic and functional analyses we aim to understand how these genetic manipulations affect microglial responses to AD-associated insults. We have also developed a protocol for integrating microglial progenitors into oligo-cortical organoids, enabling us to study microglia in the context of other brain cells including neurons, astrocytes and oligodendrocyte lineage cells. Preliminary characterization of these complex organoids by immunofluorescence, single-cell transcriptomics and electrophysiology suggests that microglia integrated in a 3D neural environment acquire the highly ramified morphology of resting microglia in the endogenous brain and contribute to the synchronization of neuronal activity. This provides us with a powerful model in which to assess the impact of key microglial genetic variants in cell-population-wide analyses.

Keywords: Alzheimer's disease, Microglia, Organoids

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APOLIPOPROTEIN E GENOTYPE SPECIFIC SYNAPTIC STABILITY IN IPSC-DERIVED NEURONS

Brown, Kevin C. - *Biology, California State University, San Marcos, CA, USA*
Verduzco, Ana - *Harold L Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA, USA*
Cline, Hollis - *Harold L Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA, USA*

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative condition that starts with synapse loss and leads to cognitive decline. Apolipoprotein E (APOE) is the main genetic risk factor for AD. The allelic variants (E2, E3, & E4) demonstrate different probabilities regarding AD risk: E2 being protective, E3 relatively neutral, and E4 increasing the probability for AD in correlation with an increase of AD-linked neuropathological markers in the brain. Previous work done by Lin et al. 2018 shows that APOE4 iNs yield higher synaptic puncta, at a single time point, compared to APOE3 iNs, suggesting APOE4 genotype neuronal cultures exhibit enhanced and early maturation. Here, we are interested in studying APOE genotype specific iPSC-derived neurons over time to better understand synaptic dynamics in an AD risk model. We will be performing a time-course tracking of pre- and postsynaptic markers (Synaptophysin and Homer-1, respectively) by using transfected reporters and immunostaining on 3 APOE expressing cell-lines: one parental APOE3, one parental APOE4, and one genetically modified (isogenic) APOE3 to APOE4. This allows us to build out a synaptic stability characterization timeline, better understand the localization and trends of overexpressed synaptic reporters, and analyze the effects of APOE expression on neuronal morphology and synaptic dynamics in iPSC-derived neurons differentiated over 31 days. We have integrated alternate steps in the differentiation protocol that allow us to re-plate, freeze, and bank early iNs, which facilitates normalizing cell density across cell-lines for the analysis of synaptic dynamics. Preliminary data shows that there is a 74% post-thawing survival rate and healthy neurite growth in the banked iNs through day 17. We hope to gain information on synaptic stabilization in APOE-specific iNs to better understand how APOE genotype might contribute to risk for Alzheimer's Disease.

Keywords: Neurons, Alzheimer's Disease, APOE

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ALLELE-SPECIFIC INACTIVATION OF DOMINANT NEGATIVE FUS MUTATIONS

Nevin, Zachary - *GIDB, Gladstone Institutes, San Francisco, CA, USA*
Matia, Madeline - *GIDB, Gladstone Institutes, San Francisco, CA, USA*
Conklin, Bruce - *GIDB, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Mutations in the nuclear RNA-binding protein "Fused in Sarcoma" (FUS) cause approximately 5% of all genetic cases of ALS and FTD and result incurable early-onset dementia and rapid motor neuron degeneration. Over 60 dominant pathologic FUS mutations have been reported, but strong genetic and experimental evidence demonstrate that FUS is haplosufficient. We have identified spCRISPR-Cas9 gRNAs that target two naturally occurring, non-pathogenic single nucleotide polymorphisms (SNPs) in exons 3 and 4 of FUS. Editing either of these SNPs allows for specific inactivation of any mutant FUS allele while leaving the normal allele intact. Due to the high incidence of these SNPs in human populations, optimization of just 4 different gRNAs could treat up to 64% of FUS-FTD/ALS patients. To study FUS pathology in a clinically-relevant system, we generated a panel of human iPSC lines and iPSC-derived motor neurons (iMN) consisting of an isogenic series of FUS mutations (Prion-like domain, C-terminal truncation, and Nuclear localization signal) engineered into the KOLF control background (which is heterozygous at both SNP loci), two independent patient lines harboring FUS mutations, and wild-type controls. Under arsenic-induced cell stress, all cell lines display cytoplasmic stress granules, but only lines with a mutation affecting the C-terminal NLS demonstrate mislocalization of FUS to these granules. Independent editing of each allele of each SNP in iPSCs demonstrates high editing efficiency and specificity of gRNA targeting SNP3-C, SNP3-A, and SNP4-C alleles, but poor editing of SNP4-T. Subsequent differentiation of iMNs of each edited mutant allele shows resolution of FUS mislocalization under stress. These studies demonstrate a potential therapeutic approach for FUS-FTD/ALS via SNP-targeted editing. We are now exploring alternate gRNA and Cas enzymes (SaCas9, Cas12a) for improved allele specificity. We are also deep phenotyping our isogenic panel to explore how different FUS mutations lead to distinct cellular pathologies and variable clinical presentations.

Keywords: FTD/ALS, FUS, CRISPR

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~~A REDUCTION IN THE AUTISM ASSOCIATED GENE LYSINE DEMETHYLASE 6B (KDM6B) ALTERS CORTICAL DEVELOPMENT~~

~~**Buth, Jessie E.** - *Neurobiology, University of California, Los Angeles, Van Nuys, CA, USA*~~
~~Gulesserian, Harout - *Biology, CSUN, Northridge, CA, USA*~~
~~Durrani, Amina - *Neurobiology, UCLA, Los Angeles, CA, USA*~~
~~Ishida, Taylor - *Neurobiology, UCLA, Los Angeles, CA, USA*~~
~~Turcios, Felix - *Neurobiology, UCLA, Los Angeles, CA, USA*~~
~~Novitch, Bennett - *Neurobiology, UCLA, Los Angeles, CA, USA*~~

~~Abstract: Autism spectrum disorder (ASD) is a heritable lifelong developmental disability that affects 1-2% of the population worldwide. Individuals experience challenges with social interaction, communication, and repetitive behaviors. Treatments include behavioral therapy, medications, and assistive technology focused on improving the patient's quality of life. Pathological studies of ASD brains point to changes in cortical cytoarchitecture, synapse formation/pruning, altered excitatory/inhibitory neuron balance, microglial activation, and astrocytosis, but the mechanisms of how these changes occur remains unclear. 100s of ASD-risk genes have been identified, many of which modify histones/chromatin. Lysine demethylase 6B (KDM6B) is a histone modifier that removes repressive tri-methyl marks on lysine 27 of histone 3 to promote gene activation; a heterozygous loss-of-function is associated with ASD. Previous studies have shown KDM6B is expressed in all cell types in the brain. In mice, overexpression results in upregulation of neuronal and astroglial genes and knockdown results in a microcephalic phenotype with fewer cortical neurons. KDM6B has also been implicated in inflammation, neurodegenerative disease, and cancers. As many clinical trials for neurological disorders based on mouse data fail, findings need to be validated with human models. Here, we compared the effect of KDM6B inhibition or CRISPR knockdown on brain development using human pluripotent stem cell (hPSC)-derived~~

neural progenitors (NPCs) and brain organoids. We found KDM6B inhibition in 2D NPCs differentiated to neurons maintained NPC identity and reduced neuron production. Since there were no overt changes to cell health, we hypothesized the reduction was due to a stall or delay in differentiation rather than cell death. We then assessed changes in brain organoid: (1) cell death, (2) NPC sub type formation/maintenance, (3) production of neurons and astrocytes, and (4) synapse density. As KDM6B is also known to play a role in inflammation, future studies will incorporate microglia into the organoid model and assess how knockdown in the organoid, microglia, or both effects development and maturation. These experiments provide a better understanding of ASD mechanisms and help narrow the focus of possible treatment targets.

Funding Source: This study was supported by funding provided to Jessie Buth by the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Rose Hills Foundation Graduate Scholarship Training Program.
Keywords: Autism, Brain Organoid, Cortical Development

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A PATIENT-DERIVED IPSC MODEL TO STUDY GLUTAMATE DEFICIENCY BY SHANK-3 MUTATION IN AUTISM SPECTRUM DISORDER

Caccia, Courtney - *Biology, Providence College, Smithfield, RI, USA*
Berry, Tiffany - *Biology, Providence College, Providence, RI, USA*
Toth, Charles - *Biology, Providence College, Providence, RI, USA*

Abstract: Autism Spectrum Disorder, or ASD, is a range of developmental delays which causes behavioral, social, and emotional challenges in the people that it affects. A diagnosis of ASD can be given as early as 18 months old for some or much later in life for others. Typically there is no single cause of ASD but rather it is caused by a mix of genetic and environmental factors. A common genetic mutation that is highly correlated with ASD is a deletion of the SHANK-3 gene. SHANK-3 is responsible for communication between neurons as it encodes for a scaffolding protein that creates dendritic spines. Mutations or deletions of SHANK-3 are tied to some developmental delays present in ASD in 1-2% percent of cases. The lack of communication between neurons due to the SHANK-3 mutation leads to lowered levels of glutamate in cells. This research project aims to see if treating glutamatergic cortical neurons with a glutamate enhancer would lead to additional synapses being formed and therefore enhanced communication. This hypothesis will be tested by setting up cortical neuron/astrocyte co-cultures from SHANK3 mutated iPSCs and an isogenic control line. Validation experiments were performed using qPCR and immunofluorescence to confirm accurate cell types. The co-cultures developed from these iPSCs will allow for a comparison of glutamate levels between mutant and control lines. Both lines will be treated with riluzole, which is a glutamate enhancer. Glutamate levels will be measured using ELISA to determine if glutamate in the ASD patient line has the potential of being corrected. The goal of this experiment is to increase glutamate levels of cells in patients with ASD which could restore normal neural communication, combating some developmental delays present in those affected.

Funding Source: Providence College Undergraduate Research Grant
Keywords: Autism Spectrum Disorder, Human iPSC, Glutamate

TOPIC: PANCREAS

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TRANSCRIPTIONAL REGULATORS OF MATURATION IN STEM CELL-DERIVED B-CELL

Augsornworawat, Punn - *Biomedical Engineering, Washington University, Saint Louis, MO, USA*
Millman, Jeffrey - *Medicine, Washington University in St. Louis, MO, USA*

Abstract: Pancreatic islets are responsible for secreting hormones to regulate blood glucose homeostasis in the body. Within the islet, the β cells secrete insulin in response to elevated glucose levels. While stem cell-derived β cells are capable of such features, they do not recapitulate β cells in native primary islets as they produce and secrete lower amounts of insulin. Herein, we use single-cell sequencing and newly developed bioinformatics tools to interrogate the transcriptional networks by comparing β cells that are stem cell-derived and β cells that are from cadaveric human islets. We identified genes associated with inflammatory signals such as FOS and JUN signaling to be associated with mature β cells. Using pseudo-time ordering analysis, we observed a strong correlation and coherence of these genes to be co-expressed with known β-cell markers such as MAFA, UCN3, G6PC2, and IAPP. We performed gene editing on stem cell-derived β cells to manipulate the expression of JUN to measure changes in β cell maturation. Previously, we have reported a strategy to mature human stem cell derived β cells by transplantation in mice. By transplanting these cells that carry JUN knockdown, the β cells have defective insulin secreting capabilities when compared to the unedited control. Single-cell RNA sequencing revealed that these β cells lack the expression of key mature β-cell genes such as IAPP, UCN3, and G6PC2. Collectively, these experiments and analyses led us to establish the importance of FOS/JUN signaling in β-cell development and maturation.

Keywords: Diabetes, Beta Cells, Maturation

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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EX VIVO CHIP BASED NEURAL TUBE ORGANOIDS FOR FUNCTIONAL SCREENING OF STEM CELLS FOR IN UTERO TREATMENT OF MYELOMENINGOCELE

Wyle, Yofiel B. - *Molecular Physiology, University of California, Davis, Sacramento, CA, USA*
Karzbrun, Eyal - *Department of Physics, University of California Santa Barbara, CA, USA, Kavli Institute for Theoretical Physics, Santa Barbara, CA, USA*
Khankhel, Aimal H. - *Biomolecular Science and Engineering, University of California, Santa Barbara, CA, USA*
Megale, Heitor C. - *Department of Physics, University of California, Santa Barbara, CA, USA*
Glasauer, Stella M. K. - *Department of Molecular, Cellular, Developmental Biology, University of California, Santa Barbara, CA, USA, Neuroscience Research Institute, University of California, Santa Barbara, CA, USA*
Britton, George - *Systems Synthetic and Physical Biology Program, Rice University, Houston, TX, USA*
Warmflash, Aryeh - *Department of Biosciences, Rice University Houston, TX, USA, Department of Bioengineering, Rice University, Houston, TX, USA*
Kosik, Kenneth S. - *Department of Molecular, Cellular, Developmental Biology, University of California Santa Barbara, CA, USA, Neuroscience Research Institute, University of California Santa Barbara, CA, USA*
Siggia, Eric D. - *Center for Studies in Physics and Biology, The Rockefeller University, New York, NY, USA*
Shraiman, Boris I. - *Department of Physics, University of California Santa Barbara, CA, USA, Kavli Institute for Theoretical Physics, Santa Barbara, CA, USA*
Streichan, Sebastian J. - *Department of Physics, University of California Santa Barbara, CA, USA, Biomolecular Science and Engineering, University of California Santa Barbara, CA, USA*
Farmer, Diana L. - *Department of Surgery, University of California Davis, Sacramento, CA,USA*

Abstract: There is a wealth of preclinical data demonstrating the safety and therapeutic efficacy of early gestation placenta-derived mesenchymal stem cells (PMSCs) for neural tube defect (NTD) repair. In an ovine model of myelomeningocele (MMC), the most severe form of spina bifida, PMSC treatment functionally cured paralysis 75% more than the standard in utero repair. The FDA has recognized the promise of PMSC therapies for treating MMC, granting phase I approval for the Cellular therapy for in Utero Repair of Myelomeningocele (CuRe) trial. The CuRe trial aims to revolutionize MMC treatment by combining a PMSC product with a modern, in utero surgical repair. Briefly, PMSCs are seeded onto a commercially available dural graft extracellular matrix (ECM) before the PMSC-ECM patch is surgically attached to the dura of the fetus. After the patch is secured, the fetal skin is closed, amniotic fluid is replaced, and the uterus and abdominal skin of the mother are closed. While there are promising preliminary results, an unmet need for the CuRe trial is a disease-specific screening assay that can accurately and robustly identify optimal cell lines for fetal repair. Currently, the team relies on a neuroprotective assay using indirect coculture of PMSCs with neuroblastoma cells cultured on a 2D monolayer after staurosporine treatment. This assay fails to recapitulate the neural tube-specific cell types nor the structural and environmental elements of NTDs, resulting in limited translatability. To address this unmet challenge, we will be leveraging a recently established chip-based neural tube organoid system capable of i) generating self-organizing, anatomically accurate human neural tubes with 90% fidelity and ii) reproducibly simulating primary NTDs using pharmacological approaches. This organoid system will provide a high-throughput, disease-relevant screening platform to identify optimal PMSC lines prior to fetal transplantation. This project aims to demonstrate that advancements in bioengineering can be leveraged immediately to enrich efficacy and reduce off-target potential in current clinical trials.

Keywords: Neural Tube Organoid, Placental MSC, Spina Bifida

TOPIC: PLURIPOTENT STEM CELLS

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SODIUM CHLORIDE AFFECTS CARTILAGE ASSOCIATED MARKER EXPRESSION DURING IN VITRO CHONDROGENESIS FROM INDUCED PLURIPOTENT STEM CELL

Lee, Kijun - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*
Son, Byeong Gwan - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*
Ryu, Young-Woo - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*
Choi, Si Hwa - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*
Rim, Yeri Alice - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*
Ju, Ji Hyeon - *Catholic iPSC Research Center, The Catholic University of Korea, Seoul, Korea*

Abstract: Induced pluripotent stem cells (iPSC) have the ability to differentiate into various lineages, and are one of the sources of stem cells that have been studied in recent years. Upregulation of osmotic pressure has been found to have a positive effect in the differentiation process of chondrocytes and is known to affect inflammation and hypertrophy, but the results of studies on cartilage differentiation in 3D cell culture have not been accurately performed. Sodium chloride (NaCl) has been reported in several studies in terms of inflammation and cartilage formation in osteoarthritis and various cartilage-related diseases. We aimed to investigate whether sodium chloride could help cartilage formation in the 3D cartilage differentiation process of iPSCs. To create a high osmotic pressure environment, a cartilage differentiation culture medium with a high osmotic pressure environment of up to 420 mOsm was prepared using NaCl as well as mannitol, one of the sugar alcohols. Embryoid body-outgrowth cells (EBOGCs) derived from iPSCs were differentiated in a chondrogenic differentiation medium supplemented with different concentrations of NaCl or mannitol for 4 weeks, and inflammatory markers including cartilage markers and hypertrophy markers were evaluated. In this paper, NaCl showed anti-inflammatory effects, but mannitol had no effect on inflammatory markers. Depending on which osmotic agent is used under the same osmotic pressure, various effects on inflammatory markers including hypertrophy, bone, and cartilage-related markers can be obtained. Appropriate concentration and use of NaCl during chondrogenesis may play an important role in cartilage regeneration or the inflammatory milieu of joints. Our findings suggest that it is important to properly use high-concentration osmotic signals for iPSC cartilage formation,

which is potentially applicable for cell therapy in patients with cartilage lesions.

Keywords: Induced pluripotent stem cells, chondrogenesis, osmolarity

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MODELING BIPOLAR DISORDER USING HUMAN IPSC-DERIVED BRAIN ORGANOIDS

Attili, Durga - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Yocum, Anastasia - *Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA*
Tran, Henry - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
MacDonald, Matthew - *Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA*
Salisbury, Ryan - *Department of Psychiatry, University of Pittsburgh, PA, USA*
Ruiz, Rebekah - *Department of Psychiatry, University of Pittsburgh, PA, USA*
McInnis, Melvin - *Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA*
O'Shea, Sue - *Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*

Abstract: Bipolar disorder (BD) is a serious neuropsychiatric condition characterized by recurring episodes of mania and depression. While heritable, no single gene or gene product has been shown to cause BD. GWAS have identified a SNP, rs1006737, in the CACNA1C gene as one of the most common associations with BD. Here, we generated induced pluripotent stem cells (iPSCs) from four BD patients carrying the CACNA1C gene mutation and from four undiagnosed control individuals. These were differentiated into three-dimensional (3D) forebrain cortical organoids. Immunohistochemical analysis and transcriptional profiling of organoids at 30, 60 and 90 days of differentiation as well as proteomic and phosphoproteomic profiling of the organoids at 30, 90 and 270 days were carried out. There were morphological alterations in the BD organoids and RNA-seq analysis identified changes in the expression of neuronal excitability-related genes, while the proteomic and phoshoproteomic data revealed differences in signaling networks over time in BD. BD samples also delay expression of neuronal markers, suggesting that there may be cell cycle alterations that affect neural lineage differentiation in these cells. Current investigations are focused on examining the effects on differentiation of manipulating pathways involved in neural development. This study demonstrates that stem cell derived models can be used to uncover novel phenotypes associated with neuropsychiatric disorders.

Funding Source: Supported by NIMH grant U19 MH1064334
Keywords: BRAIN ORGANOIDS, BIPOLAR DISORDER, MODELING IPSC-DERIVED

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IDENTIFYING REGULATORS OF PARENTAL IMPRINTING BY CRISPR/CAS9 SCREENING IN HAPLOID HUMAN EMBRYONIC STEM CELLS

Bar, Shiran - *CSB, Stanford University, Stanford, CA, USA*
Vershkov, Dan - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Keshet, Gal - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Lezmi, Elyad - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Meller, Naama - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Yilmaz, Atilgan - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Yanuka, Ofra - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*
Nissim-Rafinia, Malka - *Department of Genetics, The Hebrew University of Jerusalem, Israel*
Meshorer, Eran - *Department of Genetics, The Hebrew University of Jerusalem, Israel*
Eldar-Geva, Talia - *IVF Unit, Division of Obstetrics and Gynecology, Shaare Zedek Medical Center, Jerusalem, Israel*
Benvenisty, Nissim - *The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Inheritance of epigenetic marks from parents to offspring is known to occur in many organisms. However, in mammals, this is largely restricted due to the global erasure of DNA methylation during preimplantation development. The most well-known exception to such epigenetic reprogramming is parental imprinting. In imprinted loci, DNA methylation marks that are established differentially between oocyte and sperm are inherited to the embryo and maintained in somatic tissues, leading to monoallelic expression. Nevertheless, the mechanisms that protect these differentially methylated regions from erasure are largely obscure, especially in humans. Human embryonic stem cells (hESCs) are a valuable tool for exploring molecular mechanisms that are associated with human development and pluripotency. In this study, we used parthenogenetic human embryonic stem cells (pESCs), which lack a paternal allele. Paternally-expressed genes (PEGs) are silenced in these cells, thereby simplifying the interrogation of imprinting. To identify candidate proteins that are required for preserving imprinting, we established a genome-wide CRISPR/Cas9 screen in haploid pESCs and focused on the expression of the imprinted gene PEG10 as a proxy. Following validation of selected candidates, we could establish the involvement of ATF7IP and ZMYM2 in maintaining DNA methylation, repressive histone modification and monoallelic expression at the PEG10 locus. We further demonstrated that ATF7IP is required for the maintenance of additional imprinted loci. ATF7IP was shown to be essential for establishing SETDB1-mediated H3k9me3. However, its function was mostly studied in cancer cells and its role in hESCs was not explored. Therefore, we next focused on the global effects of ATF7IP KO in parthenogenetic and biparental hESCs, which showed a significant upregulation of genes which are specifically expressed in the male germline and are associated with sperm functions. Collectively, by conducting a genome-wide loss-of-function screening in parthenogenetic hESCs we identified novel factors that are required for imprinting preservation. Our results reinforce an important role for histone modification in imprinting regulation and suggest a link between silencing of imprinted and germ-cell genes.

Keywords: Parental Imprinting, Human Pluripotent Stem Cells, DNA Methylation

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GENERATION OF NOTCH3 MUTANT-IPS CELL LINE BY CRISPR-CAS9 TO INVESTIGATE THE ENDOTHELIAL DYSFUNCTION IN CADASIL

Aygar, Sema - *Stem Cell and Regenerative Biology, Harvard University/Stem Cell Institute, Boston, MA, USA*
Daheron, Laurence - *Stem Cell and Regenerative Biology, Harvard University Stem Cell Institute, Cambridge, MA, USA*

Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is one of the Mendelian monogenic diseases that occurs in 2-5 people per hundred thousand, characterized by NOTCH3 mutation and cerebral small vessel pathology leading to vasoconstriction, compromised endothelial reactivity, and blood-brain-barrier (BBB) permeability. Most studies have focused on the impairment of smooth muscle cells. Here we propose to investigate the role of endothelial cells in CADASIL. The impaired permeability of vascular endothelium may be the cause of vascular smooth muscle cell destruction in CADASIL. Because the endothelial cells are the sole sites of the exchange of substances between the blood and the brain, it was emphasized that the transendothelial exchange/endothelial permeability could play an important role. While the clinical characteristics of CADASIL as a vascular disease of the brain are well defined, there is still a lack of understanding of the molecular mechanisms underlying the vasculopathy, and currently no effective treatment for this disorder. Novel mechanisms underlying CADASIL vasculopathy showed that the inhibitors of RhoA/Rho kinase (ROCK) attenuate vascular dysfunction. The main purpose of this study is to investigate the effects of ibuprofen, a nonsteroidal anti-inflammatory drug acting via inhibition of cyclooxygenases and ROCK, on endothelial cells. First, we created an in-vitro model for CADASIL using iPS cells. We selected the Arg133Cys mutation, which is the most common Notch3 mutation in CADASIL. We introduced this mutation in the PGP-1 iPS line with the CRISPR/Cas9 technology. After screening 96 clones, we obtained 10 homozygous and 1 heterozygous clone for the Notch 3 mutation. We also differentiated the PGP1 cells into endothelial cells. After inducing primitive streak and mesoderm differentiation, the cells were further differentiated into endothelial cells in presence of VEGF and BMP-4. Endothelial cells were then sorted by CD31 beads and confirmed by cobblestone morphology, matrigel tube-formation assay, and expression of endothelial-specific markers. Next, both mutant and wild-type cells will be differentiated to endothelial cells to investigate the effects of ROCK inhibitor, Ibuprofen on this CADASIL-endothelial cell model.

Funding Source: The Turkish Fulbright Commission-Fulbright Postdoctoral Program
Keywords: Induced Pluripotent Stem Cell (iPSC), CRISPR/Cas9, Disease model

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GENERATION OF ISOGENIC TRISOMY 21 IPSC RESOURCES

Bhattacharyya, Anita - *Waisman Center, Department of Cell and Regenerative Biology, University of Wisconsin, Madison, WI, USA*
Yin, Yingnan - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Turner, Kristi - *Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, VA, USA*
Soref, Cheryl - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Strand, Bennett - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Xu, Ke - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Sorci, Isabella - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Held, Dustie - *Waisman Center, University of Wisconsin-Madison, Madison, WI, USA*
Jackson-Cook, Colleen - *Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, VA, USA*

Abstract: Human induced PSCs (iPSCs) from patients offer a novel model system to reveal cellular and molecular events underlying normal and abnormal development, as well as a means to better understand human disease pathogenesis. Studies using iPSCs are especially valuable for enhancing our understanding of complex traits associated with Down syndrome (DS) that cannot be fully recapitulated in animal models. iPSCs derived from somatic cells (skin or blood cells) of individuals with DS are an especially valuable model system for teasing apart cellular alterations resulting from a trisomic imbalance since these cells retain disease-related features following reprogramming into iPSCs. Yet, variability in human PSCs has complicated the ability to discern phenotypes that are attributable to trisomy 21 from those arising from human variation. Variability can be addressed through the use of isogenic controls. Given that the phenotypic effects of Ts21 can involve multiple genes, isogenic iPSCs cannot simply be developed for this condition through current gene editing technology. To address the challenge of creating isogenic iPSCs for DS, cells from people with mosaicism for trisomy 21 (mDs) were used to generate isogenic iPSCs. Since people with mDs have 2 types of cells that differ only in the presence (or absence) of trisomy 21, comparisons of biological patterns present in these genetically identical (isogenic) cells provide an ideal and exceptionally powerful means for identifying trisomy 21-specific biological alterations that lead to phenotypic traits. We present the establishment of isogenic trisomy 21 and euploid iPSCs and provide these cells as a resource to the research community. The resulting isogenic iPSCs will be characterized (including donor phenotype), banked, distributed by WiCell, and made available to researchers worldwide.

Funding Source: National Institute of Child Health and Human Development grant 1R03HD092640 to AB and CJC, and funding from UW-Madison and the Wisconsin Alumni Research Foundation and a core grant to the Waisman Center from the (U54 HD090256).
Keywords: Disease modeling, Down syndrome, iPSCs

TOPIC: CARDIAC

923

INVESTIGATING THE MECHANISMS OF HYPERTROPHIC CARDIOMYOPATHY ASSOCIATED TROPONIN T (TNNT2) VARIANT R278C USING HIPSC-CMS

Maaref, Yasaman - *Biomedical Physiology and Kinesiology, Simon Fraser University, Vancouver, BC, Canada*
Jayousi, Farah - *Biomedical physiology and Kinesiology, Simon Fraser University, Vancouver, BC, Canada*
Kim, BaRun - *BC Children's Hospital Research Institute, BC*

Children's Hospital Research Institute, Vancouver, BC, Canada
Shafaattalab, Sanam - *BC Children's Hospital Research Institute, BC Children's Hospital Research Institute, Vancouver, BC, Canada*
Li, Alison Yueh - *Biomedical physiology and Kinesiology, Simon Fraser University, Vancouver, BC, Canada*
Tibbits, Glen - *Biomedical physiology and Kinesiology, Simon Fraser University, Vancouver, BC, Canada*

Abstract: Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease and often results in cardiac remodeling, increased incidence of sudden cardiac arrest (SCA), and death, especially in youth and young adults. This study aims to investigate the mechanisms of the pathological consequences of the HCM causing R278C+/- variant in TNNT2 which encodes for the cardiac-troponin T (cTnT) protein. The R278C+/- variant exhibits variable expressivity among patients despite causing little to no cardiac hypertrophy. Moreover, the R278C+/- variant significantly increases myofilament Ca2+ sensitivity and decreases the Ca2+ off-rate constant (koff) in a human cardiac reconstituted thin filament (hcRTF) model. A transcriptomic analysis was conducted on WT vs. R278C+/-TNNT2 hiPSC-CMs using Nanostring technology, in which a custom designed codeset of 251 relevant genes was used to assess the mRNA expression profiles. The data showed a significant upregulation of atrial natriuret-ic peptide (ANP), brain natriuretic peptide (BNP), HEY2, and other remodelling components in R278C vs. WT. To investigate this further, a miRNA codeset which includes more than 800 miRNAs was used and demonstrated that the R278C+/- variant exhibited a profound effect on the miRNA expression profile in comparison to its isogenic control. These differences included the upregulation of miR 133a-3p and miR-149-5p suggestive of remodeling taking place since miR-133a-3p plays significant roles in the proliferation, differentiation, hyper-trophic growth, and electrical conduction of cardiac cells. Due to the complex impact of this variant on the transcriptome, it is critical to understand the subsequent changes implicated on the proteome and contractility. To gain a novel perception regarding the intrinsic mechanisms and pathways responsible for the pathogenic cardiac remodelling seen in HCM patients, protein characterization using bottom-up Mass Spectrometry (MS) will be used. In addition, a biomechanical analysis of TnT- R278C+/- hiPSC-CMs is being conducted using atomic force microscopy (AFM). Therefore, studying the alterations in transcriptome, proteome, and function presents the opportunity to identify new therapeutic targets for this clinically challenging disease.

Keywords: Hypertrophic cardiomyopathy, human induces pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), Troponin-T (TNNT2) variants

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CHRONIC OPTICAL TACHYPACING IN 3-DIMENSIONAL ATRIAL-LIKE ENGINEERED HEART TISSUE FROM HUMAN IPSC-DERIVED CARDIOMYOCYTES

Pan, Bangfen - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
Hansen, Julius - *Sarcura GmbH, Sarcura GmbH, Vienna, Austria*
Höppner, Grit - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
Stüdemann, Tim - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
Nzelu, Chukwuemeka George Onyeka Anene - *Cardiovascular Research Institute, National University Health Systems, National University of Singapore, SSingapore*
Lee, Mick Chang Jie - *Cardiovascular Research Institute, National University Health Systems, National University of Singapore, Singapore*
Christ, Torsten - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
Foo, Roger SY - *Cardiovascular Research Institute, National University Health Systems, National University of Singapore, Singapore, Singapore*
Eschenhagen, Thomas - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
Stenzig, Justus - *Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Abstract: Chronic rapid pacing is commonly used in animal models for atrial fibrillation (AF) studies. Previously, we established optical chronic tachypacing in human engineered heart tissue (EHT) from hiPSC-derived cardiomyocytes (CM), in which channelrhodopsins were lentivirally expressed and used to trigger action potentials in EHTs. Here, we aimed to develop a simplified optogenetic approach for chronic rapid pacing of atrial-like EHTs. We created a Volvox-Channelrhodopsin-1 (VChR1) hiPSC line by CRISPR/Cas9 genome editing. The VChR1 tagged with EYFP was inserted into the AAVS1 locus. Atrial-like CMs were differentiated from hiPSC by adding 1 µM retinoic acid (RA) at the cardiac induction stage and were subsequently used to cast fibrin-based 24-well format EHTs. Compared to control-EHTs, RA-EHTs showed higher expression of atrial genes (NPPA, MYL7, KCNJ3, KCNA5, NR2F1, NR2F2 and PITX2), corresponding lower expression of ventricular genes (MYL2 and IRX4) and displayed atrial-like action potentials. In order to conduct chronic tachypacing under normal EHT culture conditions, we designed and built a flexible and straightforward optogenetic tool, composed of a custom-designed circuit board with a programmable microcontroller and 24 addressable tri-colour (RGB) light-emitting diodes (LEDs). We placed the LED board underneath the EHT culture plate and stimulated RA-EHTs starting at day 30 by 25-90 second bursts of blue light pulses (5 Hz), separated by 10 second breaks for 40 days. Paced EHTs showed a lower spontaneous contraction force (0.096±0.06 mN vs 0.184±0.03 mN, n=33/32) and released more troponin I to the medium (6806±781 pg/mL vs 1171±195 pg/mL, n=8/8) than unpaced EHTs. RNA-seq and ATAC-seq were performed to further analyze transcriptome and chromatin accessibility. Preliminary analysis argues for a specific effect of tachypacing on both transcription and chromatin accessibility. We successfully subjected atrial-like VChR1-hiPSC derived EHTs to chronic tachypacing. The effects of pacing on contractility partially resemble the changes of atria in AF. Pacing alone induces specific transcriptional and chromatin regulatory changes. In depth analysis will contribute to the understanding of excitation-transcription coupling.

Keywords: CRISPR/Cas9, Atrial-like engineered heart tissue, Optogenetics

TOPIC: EARLY EMBRYO

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LONG RANGE CIS-REGULATION KEEPS SOX2 UP, IN PLACE AND ON TIME ENSURING PATTERNING AND CELL FATE IN THE INNER EAR

Tam, Kok Ting - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Leung, Keith - *School of Biomedical Sciences, University of Hong Kong,, Hong Kong*
Chen, PeiKai - *Department of Orthopedics, University of Hong Kong - Shenzen Hospital, Shenzen, China*
Kwong, Michael - *School of Biomedical Sciences, University of Hong Kong,, Hong Kong*
Pelling, Anna - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Fritzsch, Bernd - *Department of Otolaryngology, University of Iowa, Iowa City, IA, USA*
Lovell-Badge, Robin - *Stem Cell Biology and Developmental Genetics Laboratory, Francis Crick Institute, London, UK*
Cheah, Kathryn - *School of Biomedical Sciences, University of Hong Kong,, Hong Kong*

Abstract: Sox2, is necessary to drive prosensory fate during inner ear development. Through the study of the genomes of two Sox2 hypomorphic mutants, Sox2Ysb and Sox2Lcc, we identified regulatory elements which were disrupted in the region far downstream of Sox2. These long-range elements are essential for maintaining a gradient of Sox2 expression in the early otic vesicle which is essential for prosensory cell fate. By single cell transcriptomics we found disruption of the regulatory elements in Sox2Lcc results in cell fate change in the otic vesicle from prosensory to endolymphatic-like nonsensory identity. Interestingly these regulatory elements are conserved in human and are embedded within a 4.28Mb region shown to be associated with hearing impairment. Our data reveal temporal- and spatial-specific control of Sox2 expression in otic development, which is mediated by a series of enhancers that act in developmental time and in space and are relevant to human congenital hearing loss.

Funding Source: Health and Medical Research Fund (HMRF) 05163176 General Research Fund (GRF) 761910
Keywords: inner ear, sensory development, Sox2

TOPIC: EPITHELIAL_LUNG

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DERIVATION OF LUNG DISTAL TIP MESENCHYMAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Tamai, Koji - *Respiratory Medicine, Kyoto University, Kyoto, Japan*
Sakai, Kouji - *Department of Veterinary Science, National Institute of Infectious Diseases, Tokyo, Japan*
Yamaki, Haruka - *Department of Respiratory Medicine, Kyoto University, Kyoto, Japan*
Moriguchi, Keita - *Department of Drug Discovery for Lung Diseases, Kyoto University, Kyoto, Japan*
Igura, Koichi - *Department of Respiratory Medicine, Kyoto University, Kyoto, Japan*
Maehana, Shotaro - *Department of Environmental Microbiology, Kitasato University, Kanagawa, Japan*
Suezawa, Takahiro - *Department of Drug Discovery for Lung Diseases, Kyoto University, Kyoto, Japan*
Takehara, Kazuaki - *Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, Tokyo, Japan*
Hagiwara, Masatoshi - *Department of Anatomy and Developmental Biology, Kyoto University, Kyoto, Japan*
Hirai, Toyohiro - *Department of Respiratory Medicine, Kyoto University, Kyoto, Japan*
Gotoh, Shimpei - *Department of Respiratory Medicine, Kyoto University, Kyoto, Japan*

Abstract: Mesenchymal cells, that match the tissue type, are essential for organ development. In the lung, distal tip mesenchymal cells contribute to alveolar type 2 (AT2) and alveolar type 1 (AT1) cell differentiation and homeostasis, although knowledge of their specialized features has been limited. Here, we report a method for generating human induced pluripotent stem cell (hiPSC)-derived mesenchymal cells (iMES) similar to the ones in the distal tip of the lung, which could induce human PSC-derived AT1 and AT2 epithelial lineages in organoids via epithelial-mesenchymal interaction, without the use of fetal lung fibroblasts. First, we induced mesoderm expressing NCAM, PDGFRα, and KDR, and then differentiated it into iMES expressing VIM, THY1, PDGFRα, KDR, and FOXF1. 3D co-culture of lung epithelial progenitor cells derived from the SFTPC-GFP reporter hiPSC and iMES derived from their parental non-reporter hiPSC line (201B7) produced spheroids including SFTPC-GFP+ AT2 cells. To elucidate the role of iMES in inducing AT2 cells, we compared primary fibroblasts with iMES. We generated iPSCs from human fetal lung fibroblast (HFLF) and human dermal fibroblast (HDF) by the episomal transfection of reprogramming factors. HFLF- and HDF-iPSC lines were differentiated into iMES and compared with their parental fibroblasts in the isogenic backgrounds. Through a transcriptome comparison, we found that RSPO2 and RSPO3 expressed in iMES directly contributed to AT2 cell induction during organoid formation. iMES could expand SFTPC-GFP+ cells by passage culture and scRNA-seq analysis of iMES-AOs at P2 delineated AT1, AT2, ASCL1+ cells and ciliary cells. Their trajectory inference showed that AT1, ASCL1+, and ciliary cells branched from the AT2 cell cluster, suggesting that they were derivatives of AT2 cells. Finally, we demonstrated efficient virus-growth and robust intrinsic interferon responses of iMES-AOs infected with influenza A virus (H1N1) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Keywords: Mesenchymal cell, Alveolar epithelial cell, organoid

TOPIC: EYE AND RETINA

931

ESTABLISHMENT OF AN IN VITRO CELL VIABILITY ASSAY USING FUCHS ENDOTHELIAL CORNEAL DYSTROPHY-DERIVED IPSCS

Sakakura, Saki - *Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Inagaki, Emi - *Ophthalmology, Keio University, Tokyo, Japan*
Sayano, Tomoko - *Ophthalmology, Keio University, Tokyo, Japan*
Sugai, Etsuko - *Ophthalmology, Keio University, Tokyo, Japan*
Yamazaki, Risa - *Ophthalmology, Keio University, Tokyo, Japan*
Fusaki, Noemi - *Ophthalmology, Keio University, Tokyo, Japan*
Hatou, Shin - *Ophthalmology, Keio University, Tokyo, Japan*
Negishi, Kazuno - *Ophthalmology, Keio University, Tokyo, Japan*
Tsubota, Kazuo - *Ophthalmology, Keio University, Tokyo, Japan*

Okano, Hideyuki - *Physiology, Keio University, Tokyo, Japan*
Shimmura, Shigeto - *Ophthalmology, Keio University, Tokyo, Japan*

Abstract: Fuchs endothelial corneal dystrophy (FECD) is one of the leading causes of corneal transplantation caused by progressive loss of corneal endothelial cells. Corneal endothelial cells in FECD are vulnerable to oxidative stress leading to mitochondrial dysfunction, cell degeneration and apoptosis. While apoptosis is an important aspect of FECD, previous reports have analyzed immortalized corneal endothelial cell lines, thus making evaluation of cell death difficult. We therefore aimed to establish a new in vitro FECD model to quantify cell viability in FECD cells. Induced pluripotent stem cells (iPSC) were derived from monocytes of FECD patients, which were then induced to corneal endothelial cells. Following treatment with H2O2, immunofluorescence analysis detected higher levels of cleaved caspase-3 and the DNA damage marker, γH2AX in FECD-derived corneal endothelial cells compared to control cells. Cell viability assay using Calcein-AM staining also revealed that cell viability was higher in FECD. Next, we assessed whether N-acetylcysteine (NAC) can protect FECD-derived corneal endothelial cells from cell death induced by H2O2. Calcein-AM-positive cells were more abundant with the addition of NAC compared with H2O2 only. This in vitro model of FECD can efficiently assess cell viability which may be used for future studies on the pathophysiology of FECD and drug development.

Funding Source: This work was supported by grants from Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science to Shigeto Shimmura (KAKENHI 19K09978) and Japan Cornea Society (Novartis Pharma Grants 2020) to Emi Inagaki.
Keywords: Fuchs endothelial corneal dystrophy, cell death, oxidative stress

TOPIC: KIDNEY

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HUMAN PLURIPOTENT STEM CELL-DERIVED KIDNEY ORGANOIDS FOR PERSONALIZED CONGENITAL AND IDIOPATHIC NEPHROTIC SYNDROME MODELING

Van Den Broek, Martijn - *Pathology and Pediatric Nephrology, Radboud University Medical Center, Nijmegen, Netherlands*
Jansen, Jitske - *Pathology and Pediatric Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
van den Berge, Bartholomeus - *Pathology and Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
van den Broek, Martijn - *Pathology and Pediatric Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Maas, Rutger - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Willemsen, Brigith - *Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Kuppe, Christoph - *Nephrology and Clinical Immunology, RWTH Aachen University, Aachen, Germany*
Reimer, Katharina - *Nephrology and Clinical Immunology, RWTH Aachen University, Aachen, Germany*
Di Giovanni, Gianluca - *Pathology and Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Mooren, Fieke - *Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
den Braanker, Dirk - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Parr, Naomi - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Nagai, James - *Computational Genomics, RWTH Aachen University, Aachen, Germany*
Drenic, Vedran - *NIPOKA, NIPOKA GmbH, Greifswald, Germany*
Costa, Ivan - *Computational Genomics, RWTH Aachen University, Aachen, Germany*
Steenbergen, Eric - *Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Nijenhuis, Tom - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Endlich, Nicole - *Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany*
van de Kar, Nicole C.A.J. - *Pediatric Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Schneider, Rebekka - *Cell Biology, RWTH Aachen University, Aachen, Germany*
Wetzels, Jack F.M. - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
van der Vlag, Johan - *Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Kramann, Rafael - *Nephrology and Clinical Immunology, RWTH Aachen University, Aachen, Germany*
Schreuder, Michiel - *Pediatric Nephrology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*
Smeets, Bart - *Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands*

Abstract: Recent advances in human stem cell-derived kidney organoid models have opened new avenues to accurately model podocyte injury in 3D in vitro. Podocyte injury can lead to nephrotic syndrome (NS) and incorrect filtering of the blood, leading to protein loss in the urine. The aim of this study is to develop and characterize human induced pluripotent stem cells (iPSC)-derived 3D kidney organoids as a first step in modeling idiopathic and congenital nephrotic syndrome-in-a-dish. Human iPSC were successfully cultured into kidney organoids. Immunocytochemistry showed clear nephrin and podocin expression in podocytes, surrounded by BSA-Cy5 perfusable CDH5+CD31+ endothelial capillaries. Single cell RNA sequencing showed a subpopulation of podocytes expressing collagen IV alpha 3, which is in vivo found in mature podocytes and the expression was confirmed by RNAscope. Bulk RNA sequencing showed superior expression of podocyte specific markers in organoids as compared to 2D conditionally immortalized podocytes. To model NS, the organoids were exposed to protamine sulphate (PS) or active focal segmental glomerulosclerosis (FSGS) plasma. PS-induced injury in organoids showed clear podocyte cytoskeleton rearrangements and

the induction of pNPHS1-1176 protein expression. The induced podocyte injury was rescued by heparin, illustrating dynamic signaling in 3D podocytes. The PS effect was organoid-podocyte specific as their 2D iPS-derived podocyte counterparts did not express pNPHS1-1176. Organoids exposed to active FSGS plasma for 4h showed increased granule formation, a podocyte stress marker, in NPHS1+ podocytes which was less abundant when treated with remission plasma. To model congenital NS in organoids, erythroblasts from a pediatric patient diagnosed with compound heterozygous mutations p.Arg138Gln (exon 3) and p.Asp160Tyr (exon 4) in the podocin (NPHS2) gene were successfully reprogrammed in iPSC. Aberrant localization and weak podocin expression was shown in organoids. Using CRISPR/Cas9 the exon 3 mutation was repaired and NPHS2 expression was restored. We successfully developed human iPSC-derived kidney organoids that will serve as a state-of-the-art tool to accurately study podocytopathies in a dish.

Keywords: Kidney organoids, Nephrotic syndrome, Podocytes

TOPIC: LIVER

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HUMAN CHEMICALLY DERIVED HEPATIC PROGENITORS (HCDHS) AS A SOURCE OF ORGANOIDS GENERATION FOR APPLICATION IN REGENERATIVE MEDICINE, DISEASE MODELING AND TOXICOLOGICAL TESTS

Salas Silva, Elsy Soraya - *Department of Surgery, Hanyang University, Seoul, Korea*
Choi, Dongho - *General Surgery, Hanyang University, Seoul, Korea*
Jeong, Jaemin - *General Surgery, Hanyang University, Seoul, Korea*
Kim, Myounghoi - *General Surgery, Hanyang University, Seoul, Korea*
Kim, Taehun - *General Surgery, Hanyang University, Seoul, Korea*
Kim, Yohan - *Molecular Cell Biology and Genetics, Max Planck Institute, Dresden, Germany*
Seo, Daekwan - *Psomagen Inc., University of Maryland, Maryland, MD, USA*

Abstract: In our research group, we previously reported that chemically derived hepatic progenitors (hCdHs), reprogrammed from human primary hepatocytes (hPHs) are EpCAM positive, which gives them the bipotential characteristic of differentiation to both hepatocytes and cholangiocytes. Thanks to this report indicating that hepatic organoids are generated from positive EpCAM cells, we took on the task of generating organoids derived from hCdHs, in order to demonstrate the efficiency of hCdHs in organoids generation and, in turn, in disease modeling, regenerative capacity and drug screening model. hCdHs were cultured on Matrigel with organoid medium to generate hCdHs derived liver organoids (hCdHOs). EpCAM expression in hCdHs was significantly higher than in primary liver cells, as well in organoids generation, which adopted the characteristic cystic shape and were stably expanded for more than 6 months. The hCdHO presented a high expression in stemness markers. Next, we carried out the hepatic differentiation of these organoids (hCdHO_DM) and, as expected, they presented elevated expression of hepatic markers as well as functional markers, showing characteristics similar to those of primary hepatocytes. hCdHO were transplanted into FRG mice to assess their regenerative capacity. The mice that received the hCdHO transplant survived longer than the control groups, and the markers of liver damage decreased. We also evaluated the response of hCdHO_DM and primary hepatocytes to different groups of drugs, obtaining that the damage caused vinblastine which is cytotoxic was comparable between organoids and hepatocytes. Finally, given that organoids showed sensitivity to cytotoxic drug, we developed a model of alcohol damage in organoids, where we observed that hCdHO_DM responded as we expected, increasing inflammatory cytokines, genes related to lipid synthesis, accumulation of these and therefore loss of the potential of the mitochondrial membrane. The results suggest hCdHs as a potential source of organoid generation, which in turn allow the study and evaluation of regenerative capacity, drug screening and disease modeling.

Keywords: Liver organoid, human chemically derived hepatic progenitor, HAC

TOPIC: NEURAL

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SHARED DYSREGULATION OF GLIAL DIFFERENTIATION UNDERLIES DISRUPTION OF GLUTAMATE SIGNALING COMMON TO HUNTINGTON'S DISEASE AND SCHIZOPHRENIA

Huynh, Nguyen P.T. - *Center for Translational Neuromedicine, University of Copenhagen, Copenhagen, Denmark*
Foti, Rossana - *Center for Translational Neuromedicine, University of Copenhagen, Copenhagen, Denmark*
Goldman, Steven - *Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY, USA*
Lu, Q Richard - *Department of Pediatrics, Brain Tumor Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
Osipovitch, Mikhail - *Center for Translational Neuromedicine, University of Copenhagen,, Denmark*
Vieira, Ricardo - *Center for Translational Neuromedicine, University of Copenhagen,, Denmark*
Zhao, Chuntao - *Department of Pediatrics, Brain Tumor Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Abstract: Recent studies have identified abnormalities in glial differentiation shared by several neuropsychiatric disorders, including both Huntington's disease (HD) and schizophrenia (SCZ). To assess whether a common mechanism might underlie the glial differentiation delay of these otherwise disparate disorders, we utilized comparative correlation network approaches to analyze RNA-seq data from both human glial progenitor cells (hGPCs) and astrocytes generated from pluripotent stem cells, which were derived from subjects with HD or childhood-onset SCZ. This analysis pinpointed gene sets that were preserved between HD and SCZ yet distinct from normal controls, and identified 174 highly-connected genes with the highest node strengths in the resultant disease-associated network. These top-ranked genes were enriched for synaptic signaling, and in particular for glutamate receptor signaling components (ADGRL3, GRID2), which we confirmed as significantly suppressed in both HD and SCZ hGPCs. Furthermore, we utilized gene regulatory network analysis to identify six important upstream regulators of the 174 top-ranked genes: ETS1, MYC, SOX10, OLIG2, BCL6, TCF7L2. Among them, OLIG2 and TCF7L2 are canonical transcription factors in glial development and maintenance. We further validated their

direct binding of downstream glutamate signaling components with ChIP-seq on freshly CD140-sorted hGPCs. Since OLIG2 and TCF7L2 are dysregulated in both HD and SCZ hGPCs, this may underlie both the impaired glial differentiation and glial glutamate receptivity observed in HD and SCZ. The consequent loss of activity-dependent activation of GPCs, reflecting their failure to perceive glutamatergic signals from nearby axons, may in turn yield deficient oligodendrocyte production and hence the hypomyelination noted in disorders, as well as the disrupted astrocytic differentiation and attendant synaptic dysfunction associated with each. Together, these data highlight the importance of shared, glial-intrinsic and transcriptionally-convergent molecular pathology in the genesis and phenotypic similarities of two genetically-unrelated disorders, HD and SCZ.

Funding Source: The Novo Nordisk and Lundbeck Foundations, the Adelson Medical Research Foundation, NIMH, and CHDI
Keywords: neurogenetics; glial progenitor cell, glia; astrocyte; oligodendrocyte, schizophrenia; Huntington disease

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MODELING DEAF1-ASSOCIATED NEURODEVELOPMENT DISORDER (DAND) WITH HUMAN BRAIN ORGANOIDS

Kim, Jonghun - *Genetics, Yale School of Medicine, New Haven, CT, USA*
Park, In-hyun - *Genetics, Yale School of Medicine, New Haven, CT, USA*

Abstract: Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders with several difficulties such as challenges with social interaction, communication problem, repetitive interests, and behaviors. However, the exact cause of ASD is still unknown. In fact, several different genes appear to be involved in ASD. Recent studies discovered that de novo missense variants in DEAF1 are highly associated with ASD and intellectual disability (ID) using exome sequencing. Furthermore, loss-of-function studies in animals demonstrated that DEAF1 is essential for early embryonic and neurodevelopment. However, fundamental understanding of the pathogenic mechanisms underlying DEAF1-associated neurodevelopmental disorder in human is still unclear. Brain organoid, a miniaturized three-dimensional (3D) brain-like structure which is self-organized from pluripotent stem cells (PSCs) closely mimic properties of in vivo human brain tissues, in terms of architecture, transcriptome, and function. Thus, this technology is widely expected to bridge the remaining gaps between animal models and humans. In this study, we focus on DEAF1, one of high confidence genes for ASD and ID, and try to investigate genetic susceptibility to neurodevelopmental disorders in human using human brain organoids.

Keywords: Brain organoids, Neurodevelopmental disorder, Autism spectrum disorder

941

L-CYSTEIN PROMOTES NEURAL DIFFERENTUIATION OF MOUSE JDP2-DEPLETED CEREBELLAR GRANULE CELL PROGENITORS

Ku, Chia-Chen - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan (Republic of China)*
Wuputra, Kenly - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Pan, Jia-Bin - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Lin, Chang-Shen - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
Wu, Deng-Chyang - *Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
Yokoyama, Kazushige K. - *Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

Abstract: Jun dimerization protein 2 (JDP2)-promoter CRE mice demonstarted that Jdp2-promter was mainly expressed in mouse cerebellar progenitor granule cells (PGCs) in the cerebellum. In addition, Jdp2-knock out (KO) mice showed impaired development of the tubular structure of cerebellum. The 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, an elevated level of reduced glutathione through upregulation of cystine-glutamate antiporter xCT/Slc7a11 via activation of antioxidant response elements (AREs), and decreased levels of reactive oxygen species (ROS) were detected. Overexpression of nuclear factor-E2-related factor 2 (Nrf2) and small musculoaponeurotic fibrosarcoma-K (sMafK) did not rescue ARE- promoter activity, indicating an essential role of JDP2 in inducing ARE activity. In addition, the expression level of cyclin-dependent kinase inhibitor 1 (p21Cip1) was increased, and then interaction between p21Cip1 and Nrf2 were evident 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 important role of p21Cip1 in controlling oxidative stress and apoptosis of PGCs in the absence of JDP2. This indicates the retarded maturation of Jdp2-KO PGCs to form the altered lobes. We have generated the cerebellar granule stem-like cells using the reprogramming methods and examined their neural differentiation in the presence of L-cysteine for 7-day's in vitro cultivation. The differentiation program to neuron in Jdp2-KO PGC-stem like cells was faster than that of WT PGC-stem like cells. The GABA alpha6 receptor, beta3-tubulin and calbindin-stained cells were also detected. The characterization of neuronal cell types and its antioxidation mechanism to differentiation will be discussed.

Funding Source: Ministry of Science and Technology (110-2314-B-037-141; 110-2320-B-037-028), National Health Research Institutes (EX109-10720SI), Kaohsiung Medical University (KMU-M106001; KMU-TC108A02), and (SA10803C; KMUH 110-0R86).
Keywords: Jun dimerization protein 2 (JDP2), Progenitor granule cells (PGCs), Neural differentiation

943

GRUFFI REMOVES CELLULAR STRESS FROM BRAIN ORGANOID DATASETS

Vertesy, Abel - *Juergen Knoblich's Laboratory, IMBA - Institute of Molecular Biotechnology Austria, Vienna, Austria*
Eichmueller, Oliver - *Knoblich Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna BioCenter (VBC), Vienna, Austria*
Naas, Julia - *Center for Integrative Bioinformatics Vienna (CIBIV), Max Perutz Laboratories, University of Vienna and Medical University of Vienna, Vienna Bio Center (VBC), Vienna, Austria*
Novatchkova, Maria - *Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria*
Esk, Christopher - *Knoblich Lab, Institute of Molecular*

Biotechnology of the Austrian Academy of Science (IMBA), Vienna BioCenter (VBC), Vienna, Austria
Balmaña, Meritxell - *Knoblich Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna BioCenter (VBC), Vienna, Austria*
Ladstätter, Sabrina - *Bock Lab, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*
Bock, Christoph - *Bock Lab, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria*
Von Haeseler, Arndt - *Center for Integrative Bioinformatics Vienna (CIBIV), Max Perutz Laboratories, University of Vienna and Medical University of Vienna, Vienna Bio Center (VBC), Vienna, Austria*
Knoblich, Juergen - *Knoblich Lab, Institute of Molecular Biotechnology of the Austrian Academy of Science (IMBA), Vienna BioCenter (VBC), Vienna, Austria*

Abstract: Organoids enable disease modeling in complex and structured human tissue, in vitro. Like most 3D models, they lack sufficient oxygen supply, leading to cellular stress. These negative effects are particularly prominent in complex models, like brain organoids, where they can prevent proper lineage commitment. Here, we analyze brain organoid and fetal single cell RNA sequencing (scRNAseq) data encompassing landmark publications of the field and new datasets totaling over 190,000 cells. We describe a unique stress signature found in all organoid samples, but not in fetal samples. We demonstrate that cell stress is limited to a defined organoid cell population, and present Gruffi, an algorithm that uses granular functional filtering to identify and remove stressed cells from any organoid scRNAseq dataset in an unbiased manner. Our data show that adverse effects of cell stress can be corrected by bioinformatic analysis, improving developmental trajectories and resemblance to fetal data.

Funding Source: Abel Vertesy received funding from EMBO LTF:11122019. Work in A.v.H.'s and J.A.K.'s laboratory is supported by the Austrian Science Fund FWF (SFBF78 Stem Cell, F 7803-B) and the EU Horizon 202programs no. 874769 and no. 695642.
Keywords: Brain-Organoids, Cellular-stress, Bioinformatics

945

ENDOGENOUS TAU AGGREGATION IN A 3D CULTURE MODEL OF HIPSC BY HUMAN AD BIOSPECIMEN

Maeda, Sumihiro - *Physiology Department, Keio University School of Medicine, Tokyo, Japan*
Hiramine, Hayato - *JKiC, JSR Corporation, Shinjuku, Japan*
Ishikawa, Mitsuru - *Physiology, Keio University School of Medicine, Shinjuku, Japan*
Itoh, Manabu - *JKiC, JSR Corporation, Shinjuku, Japan*
Nagashima, Ryosuke - *JKiC, JSR Corporation, Shinjuku, Japan*
Okano, Hideyuki - *Physiology, Keio University School of Medicine, Shinjuku, Japan*

Abstract: Tauopathies are neurodegenerative diseases with the intracellular accumulation of tau protein aggregates including Alzheimer's disease (AD). Discrepancies between the results of AD clinical trials and mouse models indicated the requirement of more appropriate human cell models resembling human brains. Thus, we aimed to establish 3D human iPSC-derived culture system harboring multiple cell types like brain organoids. However, the heterogeneity of brain organoids have made it difficult to establish an assay system for drug screenings. To overcome this problem, we aimed to develop a new culture system which produce homogenous 3D cultures. At first, we introduced MAPT N279K/P301S/IVS10+16 mutations into healthy donor-derived hiPSC by genome-editing technology to enhance tau pathogenesis. Then, cerebral organoids were formed from the hiPSC to induce various neural cell types. Next, the organoids were once dissociated to single cells and plated onto low attachment plates to induce cell clustering. Finally, we obtained multiple cell clusters, i.e. 3D culture model, containing various neural cells. The scRNA-seq analysis revealed that the 3D culture consisted of excitatory/inhibitory neurons, astrocytes, oligodendrocytes, and so on. After the addition of recombinant tau seeds to initiate tau aggregation, we could induce endogenous tau aggregation with filamentous form mainly in the 3D model of mutant hiPSC. Furthermore, the brain lysate of AD patients brain lysate but not of healthy control induced tau aggregation in the 3D model. In addition, we found that the triple mutation increased the expression of 4R tau which is an adult isoform of tau and could have not been expressed in brain organoids. Endogenous human tau aggregation by AD brain lysates will enable us to investigate the pathogenesis of individual AD cases like sporadic AD cases. This 3D culture model using mutant hiPSC will serve as a platform for both basic research and drug discovery targeting tau aggregation.

Funding Source: JST, JKiC
Keywords: Tau, Alzheimer's disease, Aggregation

TOPIC: PANCREAS

947

INDUCED PLURIPOTENT STEM CELLS DERIVED FROM INSULIN-RESISTANT OFFSPRING OF TYPE 2 DIABETIC PATIENTS EXHIBIT INCREASED OXIDATIVE STRESS AND LACTATE SECRETION

Memon, Bushra - *Qatar Biomedical Research Institute-Qatar Foundation, Hamad bin Khalifa University, Doha, Qatar*
Abdelaal, Ahmed - *Stem Cells, QBRI, Doha, Qatar*
Bettahi, Ilham - *HMC, Hamad Medical Corporation, Doha, Qatar*
Abdelsalam, Eman - *Sidra Medicine, HBKU, Doha, Qatar*
Suleiman, Noor - *HMC, Hamad Medical Corporation, Doha, Qatar*
Younis, Ihab - *Biological Sciences Department, Carnegie Mellon University Qatar, Doha, Qatar*
Al-Wakeel, Mariam - *QBRI, HBKU, Doha, Qatar*
Abou-Samra, Abdul-Badi - *HMC, Hamad Medical Corporation, Doha, Qatar*
Abdelalim, Essam - *QBRI, HBKU, Doha, Qatar*

Abstract: Insulin resistance (IR) is caused by both genetic and acquired factors, however, the genetic factors are not well understood. Few clinical studies on first-degree relatives of type 2 diabetic (T2D) patients, which have the highest genetic predisposition to T2D, have given insights into the role of IR in T2D pathogenesis; without fractionating the effect of acquired factors. Induced pluripotent stem cells (iPSCs) are excellent tools for disease modeling as they can retain the genetic imprint of the disease. Therefore, in this study, we aimed to investigate the genetic perturbations associated with IR in the offspring of T2D parents, by using iPSC technology. We generated iPSCs from lean, IR offspring of T2D patients as well as from insulin-sensitive (IS) subjects, who were categorized based on hyperinsulinemic-euglycemic clamp. The IS- and IR-iPSCs were fully characterized for pluripotency and had normal karyotyping. Transcriptomics

on IR-iPSCs revealed dysregulated gene networks indicating that they carry the genetic defects associated with IR that may place them at higher risk of developing T2D. L1TD1, RIF1, and the ZNF family members , ZNF195, ZNF770 and ZNF208 were downregulated in all IR-iPSCs whereas MFGE8, EGR1 and the lactate exporter SLC16A3 were upregulated. Our results highlight that the IR-iPSCs have increased oxidative stress and a heightened response to hypoxia indicated by accumulation of reactive oxygen species and a high susceptibility to H2O2-induced apoptosis. Moreover, the IR-iPSCs also had augmented levels of lactate secretion compared to IS-iPSCs under normal conditions. Interestingly, stimulation with insulin on serum-starved iPSCs resulted in a higher phosphorylation of Protein kinase B (AKT) in IR-iPSCs compared to IS-iPSCs. Furthermore, glucose uptake assay on mature hepatocytes derived from IR-iPSCs had a diminished capacity for glucose uptake in response to insulin compared to hepatocytes derived from IS-iPSCs. Overall, our IR-iPSC model can be employed for T2D modeling and drug screening studies that target genetic perturbations associated with IR in individuals with a high risk for T2D.

Keywords: Genetic predisposition, insulin resistance, oxidative stress

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

949

DIFFERENTIATION OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS INTO DOPAMINERGIC NEURON PROGENITORS

Ishmakej, Albi - *Biology, Oakland University, Rochester, MI, USA*
Kojan, Waleed - *Biology, Oakland University, Rochester, MI, USA*
Kapanowski, Michael - *Biology, Oakland University, Rochester, MI, USA*
Mazzella, Matteo - *Biology, Oakland University, Rochester, MI, USA*
Chaudhry, Rasul - *Biology, Oakland University, Rochester, MI, USA*

Abstract: Parkinson's disease (PD) is one of the most common progressive neurodegenerative disorders. It affects the dopaminergic (DA) neurons within the substantia nigra pars compacta of the midbrain. PD is characterized by the accumulation of alpha-synuclein in aggregates, called Lewy bodies, that lead to DA neuron loss. DA neurons produce dopamine, which acts as a neural messenger to coordinate body movement. Current PD therapies have side effects and only provide temporary relief to the symptomology, and do not treat the etiology of the disease. There is a need for regenerative therapies that restore cellularity and functioning of CNS. This study investigated differentiation of human umbilical cord mesenchymal stem cells (MSCs) into DA progenitors (DAPs) and their effects in a 6-hydroxydopamine (6-OHDA) PD model rat. Results indicated that MSC derived DAPs displayed neural morphology and a substantial decrease in MSC surface protein markers. They expressed neural markers, TUJ1 and Vimentin, and DA progenitor marker TH as determined by immunostaining. DAPs also upregulated several genes, TUJ1, Vimentin, and Nestin (neural); BDNF, GDNF, and NCAM (neural protection); FOXA2 and LMX1B (DAP); as determined by real-time PCR. As expected, 6-OHDA injected animals displayed reduced motor function compared to controls based on the behavioral analysis. Whereas the motor functions were significantly improved in 6-OHDA treated animals upon DAPs injections. Impeovement in the animals' motor function injected with both MSCs and DAPs was even more significant. These improvements, particularly with respect to contralateral rotations, were comparable to the apomorphine-treated animals. Immunohistological staining of the sections with the antibodies showed a significant decrease in TH positive cells in the 6-OHDA injected brain compared to the control. Further biochemical and molecular analysis of the brain in animals injected with cells will be presented.

Keywords: Regenerative Therapy, Mesenchymal Stem Cells, Degenerative Disease

TOPIC: PLURIPOTENT STEM CELLS

951

SPATIAL CONTROL OVER STEM CELL DIFFERENTIATION AND MORPHOGENESIS TO UNDERSTAND EARLY EMBRYONIC DEVELOPMENT

Sahni, Geetika - *Mechanobiology Institute, National University of Singapore, Singapore*
Arora, Aditya - *Mechanobiology Institute, National University of Singapore, Singapore*
Marchand, Marion - *Mechanobiology Institute, National University of Singapore, Singapore*
Viasnoff, Virgile - *Mechanobiology Institute, National University of Singapore, Singapore*

Abstract: Gastrulation is an early developmental process that involves the formation of the three principal germ layers and establishment of fundamental body plan of an organism. It involves complex inter- and intra-tissue interactions and coordinated morphogenetic processes and hence the ability to mimic gastrulation-like events in vitro has important applications in understanding tissue interactions and key morphogenic processes during early embryonic development. Here, we directed the formation of a structurally reproducible and highly-organized gastruloid tissue from human pluripotent stem cells (hPSC), which recapitulates architectural and cellular characteristics of early gastrulation. These include having a 3D trilaminar germ layer organization, primitive streak-like formation where the mesoendoderm cells undergo epithelial mesenchymal transition, E-cad to-N-cad switching, and basement membrane breakdown. This is accomplished by generating a growth factor-signaling center in embryoid bodies to spatially control induction of hPSCs and guide their organization into specific germ layers as they acquire their respective differentiation fate. Using live imaging, we uncovered that germ layer organization was a result of single cell differentiation followed by collective cell migration of endodermal cells in the gastruloid model. Altogether, the unprecedented control over germ layer organization and recapitulation of morphogenic processes greatly expands the potential application of 3D gastruloid model to study early embryonic development in vitro.

Keywords: Gastrulation, Human Pluripotent Stem Cells, Morphogenesis

TOPIC: EPITHELIAL GUT

977

THE TRANSCRIPTION FACTOR SNAIL REGULATES DROSOPHILA MIDGUT DEVELOPMENT

Kapoor, Ridam - *Department of Anatomy and Physiology, University of Melbourne, Australia*
Savva, Elena - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Dominado, Nicole - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Casagranda, Franca - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Siddall, Nicole - *Department of Anatomy and Physiology, The University of Melbourne, Australia*
Hime, Gary - *Department of Anatomy and Physiology, The University of Melbourne, Australia*

Abstract: Similar to the mammalian intestine, the Drosophila midgut epithelium is maintained by a population of intestinal stem cells (ISCs) which differentiate into transient enteroblasts (EBs), enterocytes (ECs) and enteroendocrine cells (EEs). Conserved transcription factors are known to regulate ISC maintenance and differentiation, including the Snail family. There are three Drosophila members, Snail (Sna), Escargot (Esg) and Worniu. Esg is required for ISC maintenance and EE differentiation, however, the role of Sna in regulating midgut development is not well-understood. We analysed sna mRNA expression levels in 7, 14 and 21-day old mated females and showed that although sna was at very low levels in young animals it increased substantially with age. Examination of a sna::GFP fusion protein transgenic line allowed us to determine that much of this increase occurred in ECs and EEs. sna expression levels also increased after epithelial basement membrane damage. Ectopic expression of Sna in ECs resulted in activation of STAT signaling and an increase in mitotic proliferation of ISCs indicating that Sna may be involved in age related dysplasia. Proliferation of ISCs induced by EC apoptosis was suppressed via EC-specific sna knockdown suggesting that Sna plays a role in regulating the response to epithelial barrier dysfunction. We did not observe any affect on ISC proliferation after ectopic expression of Sna in EE cells indicating that the ability to induce ISC proliferation is specific to Sna function in EC cells. We also generated GFP marked homozygous sna null mutant clones to examine the effect of loss of sna in ISCs. At 4 dphs (days post heat shock) sna null mutant clones were significantly larger than the controls. Similar results were observed at 10 dphs, indicating that Sna inhibits ISC proliferation. This was supported by RNA interference of sna as EsgTS, UAS-GFP, UAS-snaTRIP resulted in an increase in GFP-positive cells compared to controls. RNAi knockdown of esg resulted in an increase in sna expression suggesting that cross-regulation of Snail family members occurs in ISCs and proliferative capacity may depend on the integration of a “Snail-family” output. Our data indicate that Snail has dual roles in the Drosophila midgut and regulates ISC activity in cell intrinsic and extrinsic manners.

Keywords: Drosophila, Snail, Intestinal Stem Cells

POSTER SESSION III: ODD

3:00 PM – 4:00 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

501

A CELL CULTURE SURFACE ALLOWING FOR SERUM-FREE AND COATING-FREE CULTURE OF HUMAN MESENCHYMAL STROMAL CELLS

DiGiacomo, Josh - *Thermo Fisher Scientific, Frederick, MD, USA*
Dey, Isha - *Thermo Fisher Scientific, Bangalore, India*
Ahamed B, Rayees - *Thermo Fisher Scientific, Bangalore, India*
Singh, Kanika - *Thermo Fisher Scientific, Bangalore, India*
Fasuyi, Arafat - *Thermo Fisher Scientific, Frederick, MD, USA*
Johnson, Debra - *Thermo Fisher Scientific, Frederick, MD, USA*
Sporring, Sune - *Thermo Fischer Scientific, Roskilde, Denmark*
Dallas, Matthew - *Thermo Fisher Scientific, Frederick, MD, USA*

Abstract: Human mesenchymal stromal cells (hMSCs) are one of the most widely researched cell types in the cell therapy field. Due to their capabilities in differentiation, immune regulation, tissue repair, and cell signaling, hMSCs have found applications in therapies for a wide variety of diseases such as bone and cartilage disorders, diabetes, neurological disorders, and more. High quantities of hMSCs are required to prepare therapeutic doses and consequentially scale-up to larger cell cultures is required. Therefore, the process of culturing hMSCs needs to be efficient, reliable, and consistent in order to provide adequate quantities of cells for both research purposes and therapeutic applications. hMSCs are typically cultured under adherent conditions with extracellular matrix (ECM) coatings and/or media containing animal-derived serum. These factors reduce the efficiency and reproducibility of the hMSC culture process by introducing variable culture conditions, requiring additional time, and adding extra costs. We recently developed an enhanced Nunclon™ cell culture surface, which eliminates these issues by allowing for the adherent culture of hMSCs under both serum-free and coating-free conditions. The treatment that generates this surface can be applied to plastic cell culture vessels spanning a large range of sizes, from 96-well plates to T175 flasks. Using hMSCs derived from adipose tissue, bone marrow, and Warton’s jelly, we demonstrated that serum-free, coating-free culture on our enhanced cell culture surface allows for comparable hMSC morphology, viability, and doubling time as compared with serum-free, ECM-coated culture on a standard cell culture surface. In addition, we also demonstrated that hMSCs cultured under serum-free, coating-free conditions on the enhanced surface maintain equivalent expression of multipotency markers and demonstrate comparable differentiation potential when compared to hMSCs cultured under serum-free, ECM-coated conditions. Taken together, these results indicate that the enhanced Nunclon™ cell culture surface can play an important role in simplifying hMSC culture workflows and easing the transition to large volume hMSC culture.

Funding Source: Thermo Fisher Scientific
Keywords: Mesenchymal stromal cells, Cell culture systems, Xeno-free cell culture

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

503

CELLCOMM IDENTIFIES THE COMPLEX CELLULAR CROSSTALK THAT DRIVES HEMATOPOIETIC STEM CELL FORMATION AND MAINTENANCE IN THE AORTIC NICHE

Sugden, Wade W. - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Lummertz da Rocha, Edroaldo - *Microbiology, Immunology and Parasitology, Universidade Federal de Santa Catarina, Florianopolis, Brazil*
Kubaczka, Caroline - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Najia, Mohamed - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Jing, Ran - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
LeBlanc, Zachary - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Markel, Arianna - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
Dos Santos Peixoto, Rafael - *Microbiology, Immunology and Parasitology, Universidade Federal de Santa Catarina, Florianopolis, Brazil*
Daley, George - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*
North, Trista - *Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA*

Abstract: Hematopoietic stem and progenitor cells (HSPCs) are derived de novo from hemogenic endothelium in select arterial niches in the vertebrate embryo through a process termed endothelial-to-hematopoietic transition (EHT). Generation of patient-specific HSPCs is a goal for cellular therapeutics; however, current differentiation protocols attempting to recapitulate EHT from a defined cellular starting material largely fail to produce or expand long-lived multi-potent HSPCs in vitro. Here, we report a new algorithm, CellComm, which is designed to exploit unbiased single-cell profiling as input and employs novel computational analytics to predict the molecular crosstalk and downstream signaling pathways of complex multicellular environments. We generated high-quality scRNA-seq data from the entire microdissected aorta-gonad-mesonephros (AGM) region of murine embryos just prior to the first emergence of HSCs. Applying CellComm to our dataset, we identified key cell types, microenvironmental signals and transcriptional networks that control hematopoietic development. We harnessed zebrafish as a rapid and reliable cornerstone of our investigational pipeline to screen surface receptors and transcriptional regulators predicted by CellComm to be active in hematopoietic development by expression and function in an orthogonal vertebrate. Functional validation of these candidates both in murine explant culture and human in vitro hematopoietic differentiation models shows high conservation across species, and together provide further support for HSPC regulatory nodes identified by CellComm, centered on Stat3, Nr0b2, Ybx1 and App. Our findings reveal not only extensive crosstalk between signaling pathways and cellular regulators acting in the hemogenic niche, but striking convergence on common transcriptional regulators, indicative of a resilient developmental program that ensures dynamic adaptation to changes in the embryonic environment and functional redundancy to support HSPC specification and maintenance in vivo. Exploitation of these transcriptional and cell-cell communication findings will aid enhancement of human induced pluripotent stem cell (hiPSC)-derived hematopoietic differentiation protocols toward the production of functionally competent HSCs for therapeutic use.

Keywords: hematopoietic stem cells, endothelial-to-hematopoietic transition, zebrafish

TOPIC: HEMATOPOIETIC SYSTEM

505

PATHWAY CONSIDERATIONS FOR A NOVEL CYTOKINE COCKTAIL FOR CORD BLOOD-DERIVED CD34+ CELL EXPANSION IN AN AUTOMATED, PERFUSION MONOCULTURE MANUFACTURING PROCESS

Jones, Mark E. - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*
Nankervis, Brian - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*
Cunningham, Annie - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*
Frank, Nathan - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*
Miller, Mindy - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*
Sethi, Dalip - *I&D Department, Terumo Blood and Cell Technologies, Inc., Lakewood, CO, USA*

Abstract: The expansion of cord blood-derived CD34+ cells has been based on the use of a cytokine cocktails formulated with recombinant human stem cell factor (SCF), FMS-like tyrosine kinase 3 ligand (FLT3L), thrombopoietin (TPO) with the addition of Interleukin 3 (IL-3), interleukin 6 (IL-6), and an antagonist of the aryl hydrocarbon receptor depending on the cell proliferation and cell differentiation objectives in hematopoietic stem cell culture manufacturing. Here, we show that with the addition of a fibronectin-stromal cell derived factor 1 (Fn-SDF-1) coating of an automated PES hollow fiber bioreactor system and the addition of the recombinant glial cell-derived neurotrophic factor (GNDF), it is possible to expand cord blood-derived CD34+ cells using a 1% concentration, instead of the usual 10% concentration, of the base cytokine cocktail by concentrating cytokines in the lumen of the hollow fiber membrane over the course of an 8 day expansion process. The design of the cytokine cocktail was driven, in part, by bioinformatics pathway analysis to confirm the interactions among the cytokine constituents. For example, the addition of GDNF is shown to interact with BCL-2 which blocks the apoptosis of some cells. Furthermore, the addition Fn-SDF-1 proliferative signaling proteins have effectively replaced the need to co-culture with mesenchymal stromal cells in this Quantum® Cell Expansion System process. In the automated expansion of 3-mixed donor cell populations at an initial, thawed seeding of 2 x 106 positively selected CD34+ cells, the mean harvest cell population fold-increase was 51.0-fold and the mean doubling time was 34.9 hours. The mean CD45+CD34+ (54.3%) and CD133+CD38- (31.8%) immunophenotypes of the harvested cell population were confirmed with minimal contaminating CD3+, CD19+, CD56+ (0.5%) lymphocytes. A 14-day Methocult assay demonstrated the CD34+

clonal differentiation into GEMM-, GM-, and Erythroid-CFU lineages.

Funding Source: Terumo Blood and Cell Technologies, Inc.
Keywords: CD34+ Cell, Novel Cytokine Cocktail, Perfusion Monoculture

TOPIC: IMMUNE SYSTEM

507

WORKFLOW OPTIMIZATION OF ROTEA-XENON, A CLOSED MODULAR AND SEMI-AUTOMATED SYSTEM FOR CELL & GENE THERAPY

LEE, Sung-uk - *Cell Biology Research and Development, Thermo Fisher Scientific, Carlsbad, CA, USA*
Andronikou, Nektaria - *Cell Biology Research & Development, Thermo Fisher Scientific, Carlsbad, CA, USA*
Chaluvappa, Pushpalatha - *Cell Biology R&D, Thermo Fisher Scientific, Carlsbad, CA, USA*
Dargitz, Carl - *Cell Biology Research & Development, Thermo Fisher Scientific, Carlsbad, CA, USA*
Isaacson, Jason - *Cell Biology Research & Development, Thermo Fisher Scientific, Carlsbad, CA, USA*
Ji, yongchang - *Cell Biology Research & Development, Thermo Fisher Scientific, Carlsbad, CA, USA*
Kim, Young - *Cell Biology Research & Development, Thermo Fisher Scientific, Carlsbad, CA, USA*

Abstract: In recent years, there has been substantial progress in the development and application of cell and gene therapy. However, most clinical studies maintain the use of viral vector systems. Non-viral delivery systems are an attractive alternative due to their reduced cytotoxicity, immunogenicity, and mutagenesis as compared to viral vectors. Electroporation, one of the non-viral approaches, is particularly suitable due to its simplicity of use, ease of large-scale production and lack of specific immune response. Autologous T cell therapy process has many touch points and a very labor-intensive workflow with a lot of open processes and complexity. Due to those challenges, manufacturing practices make gene therapies difficult to scale and meet the therapeutic demand of treatments with large patient populations. Thermo Fisher Scientific has been working to integrate and optimize the whole process into one workflow to better serve the cell therapy industry. The Gibco CTS Rotea system is a flexible/efficient system that can isolate the cells with high viability. The newly launched Gibco CTS Xenon Electroporation System also offers reliable cell therapy development and manufacturing with high cell viability and/or recovery during the ex vivo genetic modification step. By combining Rotea and Xenon systems together into one workflow, this closed, modular, and semi-automated system will help to overcome some of the challenges and ultimately get therapies to patients faster. Furthermore, this system can be applied to different types of immune/stem cells, which trends show to be increasing in the treatments of various indications. Rotea-Xenon Workflow On Day0, human PBMC were isolated from Leukopak by Rotea. PBMC were activated with anti-CD3/CD28 dynabead for 2 days. On day2, the activated T-cells were debeaded and washed/concentrated by Rotea for EP cell density at 40-50 million cells/mL. Then, those cells were electroporated with Xenon by targeting a CAR construct to the TRAC locus with CRISPR/Cas9. On Day5 (3 days post-EP), the cells were analyzed by Attune flow cytometry and Vi-Cell, and then, they were either expanded further or cryopreserved for any other downstream application(s). % Knockout (KO) of TCRab and % expression of CAR via knock-in (KI) are > 90% and 20-50%, respectively. Viability is > 85% 3 days post-EP.

Keywords: Non-Viral, Electroporation, T cell therapy

TOPIC: KIDNEY

509

AN ENGINEERED IN VITRO KIDNEY MODEL BY INTEGRATION OF ELECTROSPINNING AND STEM CELL DIFFERENTIATION TECHNOLOGIES

Mou, Xingrui - *Biomedical Engineering, Duke University, Durham, NC, USA*
Shah, Jessica - *Biomedical Engineering, Duke University, Durham, NC, USA*
Bhattacharya, Rohan - *Biomedical Engineering, Duke University, Durham, NC, USA*
Kalejaiye, Titilola - *Biomedical Engineering, Duke University, Durham, NC, USA*
Sun, Bowen - *Mechanical Engineering and Materials Science, Duke University, Durham, NC, USA*
Hsu, Po-Chun - *Mechanical Engineering and Materials Science, Duke University, Durham, NC, USA*
Musah, Samira - *Biomedical Engineering, Duke University, Durham, NC, USA*

Abstract: The kidney is an essential organ for the maintenance of health and homeostasis through filtration of blood and production of hormones to regulate organ function. Kidney disease has rapidly become a public health crisis as a growing number of patients develop the disease and subsequently progress to end stage organ failure. Efforts to study the etiology and progression of kidney diseases include the application of two-dimensional (2D) cell culture plates and animal models. However, challenges remain as 2D cell culture systems lack the three-dimensional (3D) tissue structure and organization that play a key role in mediating cellular crosstalk and cell-matrix interactions. Furthermore, animal models cannot appropriately recapitulate human physiology due to species-specific differences and divergent developmental and cell signaling pathways. To overcome these limitations, we aim to engineer a degradable biomaterial membrane-based 3D in vitro system that mimics the structure and function of the human kidney glomerulus. The human kidney glomerulus consist of a network of capillaries that serve as the primary site for blood filtration, and has been shown to be a key target of many kidney diseases. Specifically, we are developing a highly porous electrospun biomaterial matrix coupled with microfluidic systems to mimic the human kidney glomerular basement membrane. We integrated these engineered scaffolds with our previously reported method for the derivation of human kidney podocytes from stem cells coupled with an endothelial cell differentiation protocol. The resulting in vitro system comprises two different kidney cell layers (podocytes and endothelial cells) separated by a thin electrospun membrane, which could be degraded and replaced by the glomerular basement membrane matrix secreted by the cells. The resulting engineered model provides a physiologically relevant platform for mechanistic studies and therapeutic discoveries for human kidney diseases.

Funding Source: Duke SMIF, Duke Incubation Fund, Whitehead, Burroughs Wellcome Fund, George O'Brien Kidney Translational Center, The International Foundation for Ethical Research, Chair's Research Award from Department of Medicine at Duke.

Keywords: Electrospun silk fibroin, Glomerular filtration barrier, Organ-on-chips

TOPIC: NEURAL

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UTILIZING ENGINEERED CHONDROITINASE ABC TO ENABLE NEUROREGENERATION AFTER STROKE

Letko-Khait, Nitzan - *Chemical Engineering & Applied Chemistry, University of Toronto, ON, Canada*
Zuccaro, Sabrina - *Chemical Engineering & Applied Chemistry, University of Toronto, ON, Canada*
Shoichet, Molly - *Chemical Engineering & Applied Chemistry, University of Toronto, ON, Canada*

Abstract: Following injuries to the central nervous system (CNS), such as spinal cord injury and stroke, astrocytes become reactive and deposit large quantities of proteoglycans that form a glial scar. The scar is essential to prevent the spread of the injury to healthy tissue at the acute phase after stroke, while at the chronic phase, it limits the regenerative capacity of the tissue. Cell transplantation was proposed to regenerate and repopulate the damaged tissue, demonstrating improved functional behavior in pre-clinical studies, yet cell survival and integration remain poor. The degradation of the glial scar was therefore suggested as a therapeutic strategy after CNS injuries to improve local plasticity and promote axonal regrowth and cell integration. The bacterial enzyme chondroitinase ABC (ChABC) can degrade chondroitin sulfate proteoglycans, a major component of the glial scar, and has been proposed as a therapeutic strategy for multiple disorders. Yet, its inherent thermal instability, characterized by a rapid loss of activity at physiological temperatures and pH, limits its therapeutic potential. Moreover, continuous delivery is needed to obtain significant improvements after stroke. To address the need for a sustained local release of the enzyme, we developed an affinity-controlled delivery platform composed of an injectable cross-linked methylcellulose hydrogel modified with a Src homology 3 (SH3) binding peptides. The enzyme was expressed as a fusion protein with a SH3 domain. To address the intrinsic instability of ChABC, we designed a thermostable mutant of ChABC (ChABC-37-SH3) using computational remodeling. ChABC-37-SH3 demonstrated a 6.5 times higher half-life than the native enzyme, with a higher melting temperature, and increased activity for its substrate. Affinity-controlled release from the hydrogel was achieved for at least 7 days in vitro, and the released enzyme retained its catalytic activity. We demonstrated the efficacy of our minimally invasive delivery system in vivo, in an endothelin-1-induced stroke injury model in rats, where we focused on the effect of ChABC-37-SH3 on the glial scar and tissue regeneration.

Funding Source: We are grateful to NSERC and CIHR from CHRP for funding this research.
Keywords: Ischemic stroke, tissue regeneration, Glial scar

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A HUMAN PLURIPOTENT STEM CELL-DERIVED ORGANOID MODEL FOR RECAPITULATION OF CENTRAL NERVOUS SYSTEM (CNS) BARRIER AND FLUID SECRETION FUNCTIONS OF THE CHOROID PLEXUS

Knock, Erin - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Chew, Leon - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Eaves, Allen - *Research and Development, STEMCELL Technologies Inc, Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: The choroid plexus plays a critical role in forming the blood-cerebrospinal fluid (CSF) barrier and is responsible for generating CSF in the central nervous system (CNS). The blood-CSF barrier functions to restrict entry of harmful pathogens but also therapeutics into the CNS. Recently, a human pluripotent stem cell (hPSC)-derived organoid model of the choroid plexus was developed as a tool for biomarker discovery and blood-CSF permeability assays. Here we present data from our STEMdiff™ Choroid Plexus Organoid Differentiation Kit, based on the above publication, for generating CNS barrier-forming organoids. Single-cell suspensions of hPSCs were cultured in 96-well round-bottom plates in embryoid body formation medium for 5 days at 37°C. The aggregates were switched to induction medium for 2 days (days 5 - 7), then embedded in Corning® Matrigel® and grown in expansion medium for 3 days (days 7 - 10). The culture medium was then switched to differentiation medium for 5 days (days 10 - 15). On day 15, medium was switched to maturation medium. Cystic fluid was extracted at ~day 50 for analysis and the barrier function was tested using low-molecular weight FITC-dextran. At ~day 30, choroid plexus organoids were observed to generate large cysts (> 70%, 112/144 organoids, n=6 hPSC lines) and exhibited upregulation of choroid plexus markers (TTR, CLIC6, and AQP-1) and downregulation of cortical markers (PAX6, MAP2, and FOXG1) compared to unpatterned cerebral organoids using RT-qPCR and immunostaining (n=6 hPSC lines, 3 organoids per cell line). Both clusterin and IGF2, proteins found in human CSF, were detected in the CSF-like fluid at day 50 using western blot. We further found that FITC-dextran was excluded from the cyst compartment. Our results demonstrate that STEMdiff™ Choroid Plexus Organoid Kit can generate an in vitro human model that recapitulates the CNS barrier and CSF-like secretion functions of the choroid plexus.

Funding Source: STEMCELL Technologies is a private for profit biotechnology company
Keywords: choroid plexus, cerebral spinal fluid, brain barrier

TOPIC: NT - GENERAL

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POOLED IN SITU HYBRIDIZATION ENABLES CRISPR SCREENING OF MORPHOLOGICAL PHENOTYPES VIA MACHINE LEARNING

Salick, Max R. - *Advanced Cellular Technologies, Insitro, San Francisco, CA, USA*
Sivanandan, Srinivasan - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Leitmann, Bobby - *Advanced Cellular Technologies Group, Insitro, South San Francisco, CA, USA*
Ranu, Navpreet - *Advanced Cellular Technologies Group, Insitro, South San Francisco, CA, USA*
Hao, Cynthia - *Advanced Cellular Technologies Group, Insitro, South San Francisco, CA, USA*
Chen, Owen - *Advanced Cellular Technologies Group, Insitro, South San Francisco, CA, USA*
Kim, Albert - *Automation Engineering, Insitro, South San Francisco, CA, USA*
Bisognano, John - *Data Engineering, Insitro, South San Francisco, CA, USA*
Ruggiu, Fiorella - *Computational Drug Design, Insitro, South San Francisco, CA, USA*
Sultan, Muneeb - *Computational Drug Design, Insitro, South San Francisco, CA, USA*
Lubeck, Eric - *Genentech, Genentech, South San Francisco, CA, USA*
Cesarek, John - *Automation Engineering, Insitro, South San Francisco, CA, USA*
Kaykas, Ajamete - *High-Throughput Biology, Insitro, South San Francisco, CA, USA*
Sharon, Eilon - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Chu, Ci - *Advanced Cellular Technologies Group, Insitro, South San Francisco, CA, USA*

Abstract: Pooled CRISPR screening has emerged as a powerful method of uncovering entire gene networks and modulators of critical biomarkers. Unfortunately, the current methods of CRISPR screening are only compatible with fitness or FACS-sortable phenotypes, while high-dimensional readout methods such as perturb-seq are costly and only apply to transcriptional readouts. With the recent emergence of pooled optical screening methods, perturbagens such as gene-targeting gRNAs can be amplified and directly measured via in situ sequencing while maintaining cellular structure and spatial features. This enables CRISPR screens to be coupled with a nearly limitless range of imaging assays, such as cell migration, calcium signaling, CellPaint, quantitative phase contrast, protein aggregation, multicellular/cell-cell interaction assays, and more. Here we describe an automated platform that has been developed to allow for pooled optical screening at industrial capacity, as well as multiple optical CRISPR screens done at increasing scales. We first describe a screen conducted on morphological phenotypes, in which genes targeting various core pathways were edited. We demonstrate that machine-learning based morphological analysis successfully identified and grouped these gene clusters using simply CellPaint and high-dimensional morphological readouts. In the second screen, we applied perturbations to genes with known chemical modifiers to explore whether this type of optical screen could be used to develop a morphological atlas onto which chemical perturbagens' mechanisms of action (MoA) could be mapped for any given cell type of interest. Lastly, we conducted a druggable-genome scale screen to identify both marker-based modifiers of the mTOR pathway, as well as biomarker-free clustering of gene networks using machine learning. While only a handful of pooled optical screens have been conducted so far in the field, we demonstrate the beginning of a promising new stage of CRISPR screening technology, particularly when applied to disease-relevant cell types derived from iPSCs.

Keywords: CRISPR, Pooled Optical Screening, Machine Learning

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OPTIMAL TISSUE SECTION SELECTION FOR SPATIAL TRANSCRIPTOMICS IDENTIFIES CRUCIAL MARKERS OF MAMMALIAN EMBRYONIC DEVELOPMENT

Gutgesell, Lauren - *Molecular Biology, 10x Genomics, Pleasanton, CA, USA*
Sukovich, David - *Molecular Biology, 10x Genomics, Pleasanton, CA, USA*
Yu Drennon, Tingsheng - *Sample Prep, 10x Genomics, Pleasanton, CA, USA*
Anaparthy, Naishitha - *Molecular Biology, 10x Genomics, Pleasanton, CA, USA*
Kim, Hanyoup - *Microfluidics, 10x Genomics, Pleasanton, CA, USA*
Mikhaiel, Nabil - *Microfluidics, 10x Genomics, Pleasanton, CA, USA*
Li, Dongyao - *Computational Biology, 10x Genomics, Pleasanton, CA, USA*
Russell, Spontaneous - *Product Marketing, 10x Genomics, Pleasanton, CA, USA*
Tentori, Augusto - *Microfluidics, 10x Genomics, Pleasanton, CA, USA*

Abstract: Embryonic development in mammalian models can be visualized through genomic or proteomic methods. Unfortunately, genomic methods provide whole transcriptome information but lack spatial relevance, while proteomic methods give spatial context but lack depth. Spatially resolving the transcriptome and overlapping with proteomic markers on selected tissue sections provides a comprehensive and comparative view of the model system embryo at select stages of development. The Visium CytAssist technology from 10x Genomics gives researchers the ability to transfer target molecules from FFPE tissue sections to the Visium Spatial Gene Expression slide capture arrays. The Visium FFPE assay utilizes ligated probe pairs designed to target specific sequences of RNA.These probes are then released from the tissue and are captured on the spatially-barcoded oligonucleotides on the Visium slide capture arrays. Furthermore, the Visium Spatial platform can be expanded with Feature Barcode technology using oligo-conjugated antibodies to identify corresponding, spatially resolved, protein expression. Using Visium CytAssist, users can select the tissue section slide and tissue section region that they want to align to the Visium slide capture arrays based on the morphological or pathological landmarks of interest. For this study, we chose the head and torso of the mouse embryo for the Visium multiomic spatial analysis. We identified spatially distinct regions with key markers of development and differentiation throughout the mouse embryo. For example, markers of development such as Ter-119, an erythroid specific marker, and CD45, a marker of differentiated hematopoietic cells except erythroids, appear in distinct regions of the mouse embryo. By leveraging the power of the Visium Spatial Gene Expression platform with Feature Barcode technology to identify gene and protein expres-

sion by location in the tissue and the Visium CytAssist technology to transfer nucleic acids from the tissue section of interest, researchers can optimize resources while gaining insights into cell type-specific transcriptomics overlain with tissue morphology information.

Keywords: spatial transcriptomics, multiomics, feature barcoding

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ASTRONAUT-ON-A-CHIP: HUMAN, MULTI-ORGAN PLATFORM FOR ASSESSING EXTENDED EFFECTS OF COSMIC RADIATION

Tavakol, Daniel N - *Biomedical Engineering, Columbia University, New York, NY, USA*
Nash, Trevor - *Biomedical Engineering, Columbia University, New York, NY, USA*
Kim, Youngbin - *Biomedical Engineering, Columbia University, New York, NY, USA*
Liberman, Martin - *Biomedical Engineering, Columbia University, New York, NY, USA*
O'Donnell, Aaron - *Biomedical Engineering, Columbia University, New York, NY, USA*
Graney, Pamela - *Biomedical Engineering, Columbia University, New York, NY, USA*
Fleischer, Sharon - *Biomedical Engineering, Columbia University, New York, NY, USA*
He, Siyu - *Biomedical Engineering, Columbia University, New York, NY, USA*
Teles, Diogo - *Biomedical Engineering, Columbia University, New York, NY, USA*
Yeager, Keith - *Biomedical Engineering, Columbia University, New York, NY, USA*
Summers, Max - *Biomedical Engineering, Columbia University, New York, NY, USA*
Amundson, Sally - *Radiological Research, Columbia University, New York, NY, USA*
Garty, Guy - *Radiological Research, Columbia University, New York, NY, USA*
Leong, Kam - *Biomedical Engineering, Columbia University, New York, NY, USA*
Brenner, David - *Radiological Research, Columbia University, New York, NY, USA*
Vunjak-Novakovic, Gordana - *Biomedical Engineering, Columbia University, New York, NY, USA*

Abstract: Cosmic radiation is one of the most serious risks encountered during long missions to the Moon and Mars, requiring effective measures for radiation protection. While animal models have contributed to our understanding of radiation damage and radiation protection, in vitro models consisting of engineered human tissues provide unparalleled physiological mimicry to humans exposed to galactic cosmic rays (GCR). Here, we report the use of a multi-organ-on-a-chip (OOC) platform in studies of systemic radiation consisting of engineered human tissue models of bone marrow (BM, site of hematopoiesis and acute radiation toxicity), cardiac muscle (CM, site of chronic radiation damage), liver (site of metabolism), and vasculature (barrier for transport of signals throughout system). The tissues were fabricated using human cells (primary and induced pluripotent stem cells) and tissue-specific biomaterial scaffolds, and matured individually over a period of 4-6 weeks prior to being placed into the platform. To model the effects of GCR, tissue platforms were exposed to neutron radiation at Columbia's Radiological Research Accelerator Facility. We show for the individual bioengineered human tissues within an integrated context: (1) extended cultures of a multi-OOC system containing CM, BM, liver, and vasculature after radiation exposures, (2) differential changes between acute and protracted exposures, and (3) mitigation of radiation damage using radioprotective drugs. We characterized the structural, functional, and molecular changes associated with long-duration radiation exposures. In response to 0.5 Gy acute neutron doses, we observed CD11b+ myeloid skewing of hematopoietic cells and decreased excitability of cardiac tissues. This effect was even more significant following protracted exposures, when a dosage of 0.5 Gy was distributed over a period of 2 weeks. We also showed that administration of granulocyte colony stimulating factor (G-CSF) at the time of radiation was able to prevent some of the hematopoietic injury in the BM compartment. In future work, we aim to benchmark this engineered model to data available on astronauts, animal studies, and accidental exposures to radiation on Earth.

Funding Source: NASA TRISH NNX16AO69A, NIH P41 EB027062, NSF DGE1644869
Keywords: Organ-on-a-chip, Hematopoiesis, Radiation

TOPIC: PLURIPOTENT STEM CELLS

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THERMORESPONSIVE FLASK COATING FOR IMPROVED STEM CELL HARVEST AND MAINTENANCE OF LONGEVITY AND DIFFERENTIATION POTENTIAL

Guire, Eric - *Saint Paul, MN, USA*

Abstract: Traditional cell culture techniques rely on enzymatic digestion for passaging, which introduces cell stress pathway activation, potential contamination, and a reduction in cellular viability. It has been shown that aneuploidy can develop in long-term cultures passaged enzymatically, increasing the hurdle for regulatory approval of stem cell therapies. Thermoresponsive polymer coatings can eliminate the need for enzymatic digestion by releasing cells with a polymer phase change. Current commercial options do not yet include cell culture flasks. Additionally, most of these products do not offer digestion-free passaging for strongly adherent cell lines such as mesenchymal stem cells (MSC). ISurTec has developed a thermoresponsive coating on T25 flasks for non-digestive cell release, optimized for strongly adherent cell lines. In comparison to standard culture flasks, our coating showed normal proliferation rates and metabolic activity for multiple cell lines. Our coating releases 90% of hMSC within 30 minutes. We are investigating whether hMSC have a higher retention of differentiation potential on ISurTherm than standard enzymatic digestion on TCPS as has been previously shown on thermoresponsive surfaces (Yang et al). Commercially available cord blood (CB-hMSC) and bone marrow derived hMSC (BM-hMSC) are in culture on ISurTherm-coated and TCPS control flasks for three passages (temperature reduction and enzymatic, respectively) before differentiating. Triplicate samples of each condition will be treated with osteogenic, chondrogenic, or adipogenic differentiation media per standard protocols. Differentiation potential and status will be assessed with staining, immunostaining, and/or FACS sorting. Due to the maintenance of transmembrane signaling complexes, the ECM and cell to cell junctions during passaging from thermoresponsive surfaces, we hypothesize that cells harvested from ISurTherm-coated flasks will demonstrate greater differentiation potential than those from TCPS. Additionally, we

hypothesize the CB-hMSC will show greater osteogenic differentiation and the BM-hMSC will show greater adipogenic differentiation than the TCPS controls as has been observed in previous thermoresponsive studies. This work is ongoing and will be presented for the first time at ISSCR.

Keywords: Thermoresponsive Surface for Cell Harvest, Differentiation Potential, Strongly Adherent Cell Lines

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GROWTH FACTOR PRODUCTION AND OPTIMIZATION FOR FULLY DEFINED MEDIA IN INDUCED PLURIPOTENT STEM CELL CULTURE

Pierce, Rebecca - *Biology, California State University, San Marcos, Oceanside, CA, USA*

Abstract: Currently, in academic and industry labs there is no standard medium being used to grow human induced pluripotent stem cells (hiPSCs). Each individual lab can choose between purchasing media from external suppliers or “homebrewing” media in a lab. Furthermore, these media may not be fully defined, can contain serum, and/or are proprietary formulations. These media pose a problem to scientists because they are difficult to consistently reproduce and can increase the cost of research through formulation inconsistencies or the need to purchase proprietary formulations. Additionally, media variation can cause downstream complications for other researchers trying to replicate or continue from prior experiments. I hypothesize that an optimized serum-free defined medium can increase cell growth while maintaining pluripotency and decreasing unwanted differentiation. This medium would be a cost-effective alternative to both media currently available and homebrew formulations. Towards this end, we manufactured HiDef-B8, a fully defined, serum-free medium optimized for hiPSC culture. This included in-house production of fibroblast growth factor 2 (FGF2) G3, a thermostable protein growth factor critical to the HiDef-B8 formulation, and activity validation using an NIH-3T3 fibroblast growth assay. After validation, this protein was added to HiDef-B8, for complete medium evaluation in hiPSC growth assays. Our data show pluripotency maintenance and minimal differentiation in our hiPSC cell line when grown in HiDef-B8. Low differentiation and maintained pluripotency were confirmed by microscopic observation while cell health was confirmed using a cell metabolic assay. Collectively, our data confirm that HiDef-B8 can enable cell growth in a serum-free environment while maintaining pluripotency. Our future goals are to further reduce the cost of components and enable broader use of serum-free media used in the field.

Keywords: hiPSC, serum-free, production

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COMPUTATIONALLY DEFINED AND IN VITRO VALIDATED GENOMIC SAFE HARBOUR LOCI WITH LANDING-PAD CASSETTES FOR EASY TRANSGENE EXPRESSION IN HUMAN PLURIPOTENT STEM CELLS

Autio, Matias Ilmari - *Human Genetics, GIS, Singapore*
Motakis, Efthymios - *Genome Institute of Singapore, Singapore*
Perrin, Arnaud - *Genome Institute of Singapore, Singapore*
Bin Amin, Talal - *Genome Institute of Singapore, Singapore*
Tiang, Zenia - *Genome Institute of Singapore, Singapore*
Do, Dang Vinh - *Genome Institute of Singapore, Singapore*
Wang, Jiaxu - *Genome Institute of Singapore, Singapore*
Tan, Joanna - *Genome Institute of Singapore, Singapore*
Tan, Wei Xuan - *Institute of Molecular and Cell Biology, Singapore*
Teo, Adrian Kee Keong - *Institute of Molecular and Cell Biology, Singapore*
Foo, Roger - *Genome Institute of Singapore, Singapore*

Abstract: Stable expression of transgenes is essential in both therapeutic and research applications. Traditionally, transgene integration has been accomplished via viral vectors in a semi-random fashion, but with inherent integration site biases linked to the type of virus used. The randomly integrated transgenes may undergo silencing and more concerningly, can also lead to dysregulation of endogenous genes. Gene dysregulation can lead to malignant transformation of cells and has unfortunately given rise to cases of leukaemia in gene therapy trials. Genomic safe harbour (GSH) loci have been previously suggested as safe sites for transgene integration. Criteria proposed for a putative GSH include; a set distance from coding and non-coding genes; with added separation from known oncogenes and miRNAs, and no disruption of transcriptional units or ultraconserved regions. To date, a number of sites in the human genome have been used for directed integration; however none of these pass scrutiny as bona fide GSH. Here, we conducted a computational analysis to filter sites that meet criteria for GSH loci. In addition to the safety criteria, we identified regions that reside in active chromosomal compartments in many human cell and tissue types. Our analysis yielded a final list of 25 unique putative GSH that are predicted to be accessible in multiple cell types. We used human embryonic stem cells (hESCs) and their differentiated progeny to validate stable transgene expression and minimal disruption of the native transcriptome in three of the putative GSH sites in vitro. Furthermore, we generated hESC and induced pluripotent cell lines with constitutive landing pad expression constructs targeted into the three different GSH. The generated landing pad human pluripotent cell lines allow for easy targeted over expression of genes of interest and in the pluripotent cell state or in cells differentiated to the cell type of interest.

Funding Source: This research was funded by the BMRC (Singapore) YIG 2016 (1610851033) and A*STAR CDF 2020 (202D8020) to M.I.A..
Keywords: genomic safe harbours, pluripotent stem cells, landing pad expression cassette

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A SCALABLE AND TUNABLE THERMOREVERSIBLE HYDROGEL PLATFORM FOR 3D HUMAN PLURIPOTENT STEM CELL BIOMANUFACTURING

Johnson, Hunter J. - *Bioengineering, University of California, Berkeley, CA, USA*
Chakraborty, Saheli - *Chemical and Biomolecular Engineering, University of California, Berkeley, CA, USA*
Muckom, Riya - *Chemical and Biomolecular Engineering, University of California, Berkeley, CA, USA*
Schaffer, David - *Bioengineering, Chemical and Biomolecular Engineering, Molecular and Cell Biology, University of California, Berkeley, CA, USA*

Abstract: Human pluripotent stem cells (hPSCs), with the ability to self-renew and derive all somatic cell types of the body, represent an attractive source for cell replacement therapies (CRTs),

to treat diseases of degeneration. However, clinical translation of hPSC-derived cell products is limited by the scalability of stem cell production. Diseases of cellular degeneration, such as heart failure, liver failure, and neurodegenerative disorders, can require up to 1x109 cells per patient, and considering the patient population and annual incidence, may require >1015 cells per year for the US alone. With conventional 2D cell culture, the surface area necessary to produce these cell numbers is prohibitive. Synthetic thermoreversible polymers offer an exciting approach to scalable 3D hPSC expansion and differentiation, but current PEG-PNIPA-Am based polymers suffer from batch-to-batch variability, are functionally inert, and do not comply with cGMP standards. To overcome these limitations, we have developed a fully synthetic, scalable, and tunable thermoreversible polymer to enable industrial and clinical impact of stem cell therapy. Our fully defined and reproducible polymer synthesis strategy allows the precise control of gelation temperature (12-32°C), stiffness at 37°C (100-4000 Pa), and presentation of growth factors and cell adhesion peptides (bioconjugation). Additionally, cell retrieval is simplified to cooling of the thermoreversible gel below the LCST, to liquefy the hydrogel and release the hPSC-derived product. The release results in near 100% cell retrieval at >95% viability without the need for material enzymatic degradation. The thermoreversible hydrogel enables high hPSC viability, consistent expansion (~20-fold/4-days), and pluripotency maintenance over extended culture periods (>5 passages). Additionally, we have derived potential cell therapy candidates from the three germ layers withing the hydrogel platform, including dopaminergic neurons (ectoderm), cardiomyocytes (mesoderm), and hepatocytes (endoderm) expressing characteristic functional markers and high-fold expansion. Overall, we posit this novel 3D cell culture platform may help overcome the critical bottleneck in cell manufacturing and accelerate clinical translation of hPSC-derived cell therapy candidates.

Funding Source: US National Science Foundation (to H.J.J.), the US National Institute of Health R01NS074831 (to D.V.S.) and kind gift from Dennis Chan (to D.V.S.)
Keywords: Biomanufacturing, Biomaterials, Cell Replacement Therapy

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A METHOD FOR SHEAR-BASED ADHESIVE STRENGTH EVALUATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING COLONY COMPARTMENTALIZATION

Kasai, Yusuke - *Process Engineering & Technology Center, FUJIFILM Corporation, Ashigarakami-gun, Japan*
Oba, Takahiro - *Process Engineering & Technology Center, FUJIFILM Corporation, Minamiashigara-shi, Japan*

Abstract: Adhesion of human induced pluripotent stem cells (hiPSCs) to culture substrates plays important roles to determine their biological/mechanical behavior, such as proliferation, differentiation, detachment from the substrates and death. Therefore, the adhesion strength of hiPSCs attracts wide attentions not only in fundamental studies which investigate the mechanism of hiPSCs' gene expression activated by the adhesion but also in industrial mass cell production which commonly has concerns in the mechanical detachment of hiPSCs from their substrate/microcarrier. However, there are few reports on the adhesive strength of hiPSCs due to the various obstacles of (a) difficulty in one-by-one evaluation of hiPSC colonies having a big variety of their size, (b) sensitiveness of hiPSCs against changes of culturing process, and (c) difficulty in integrating the adhesive strength evaluation system with the culturing platform. To overcome these obstacles, we propose a novel method for shear-based adhesive strength evaluation of hiPSCs. The method consists of three key components; (1) compartmentalization of hiPSC colony using a bio-compatible mask sheet which just needs an additional step of masking before extracellular matrix (ECM) coating, (2) an elastic and opened microfluidic device which can attach/detach to/from a common culture dish, and (3) an easy-to-use mechanical platform which can precisely align and combine the microfluidic device with the hiPSC-patterned dish. As a proof of the concept, we demonstrated the pattern culturing of hiPSCs having 200-um square rectangle pattern with 200-um pitch on a commercial 35-mm dish using the mask sheet. In addition, we succeeded in observation and recording of hiPSCs' detachment via a microscope under certain shear stresses. From these results, we confirmed that the proposed method enables us to quantify the adhesive strength of compartmentalized hiPSCs. This technique will lead us to understand unrevealed mechanical characteristics not only of hiPSCs but also of various types of adhesive cells.

Keywords: Human induced pluripotent stem cell, Shear stress, Adhesion

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

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WHERE ARE THEY NOW? MAPPING THE EVOLUTION OF EUROPEAN STEM CELL POLICY

Isasi, Rosario - *Human Genetics, University of Miami, FL, USA*
Assen, Lars - *UMC Utrecht, Netherlands*
Jongsma, Karin - *UMC Utrecht, Netherlands*
Ginoza, Margaret - *Human Genetics, University of Miami, FL, USA*
Fabbri, Morris - *Department of Human Genetics, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA*

Abstract: Societal attitudes and stakeholders' roles continue to change responsive to contextual factors and scientific developments. For instance, human induced pluripotent stem cell (hiPSC) research, which was once considered a practice absent of major ethical conundrums but now includes embryo modeling and chimera research which may violate the values underlying legislation, has prompted a re-evaluation of existing regulatory and governance systems. Globally, uncertainty persists concerning regulations which restrict the use of embryonic cells across the research-clinical translation continuum, but which do not seem to constrain morally contentious uses of hiPSC. In Europe (EU), policy governing stem cell (SC) research continues to be a matter of national jurisdiction resulting in heterogeneity of rules. This heterogeneity reflects the continent's diverse socio-cultural, economic and historical context. However, a 'common EU ethics' is apparent from the adoption of the 1997 European Convention on Human Rights and Biomedicine as well as the ongoing adoption of a common research funding framework, which includes legal and ethical provisions for the inescapably controversial human embryonic SC (hESC) research. Where do policies diverge and converge in EU? What are the reasons behind such variations? Are governance systems equipping researchers to act responsibly during the innovation process? How do EU policy frameworks fare against evolving international norms represented in the updated 2021 ISSCR Guidelines? This presentation addresses the evolution of SC policies in 46 EU countries. With the ISSCR Guidelines as a backdrop, we discuss central ethical and policy issues regarding contentions applications, including criteria for permis-

sibility, oversight, and enforcement mechanisms. Comparative studies provide an opportunity to promote insight into national frameworks and to foster international harmonization. The EU region represents a rich case-study as it encompasses a wide range of policy approaches present across the globe. Thus, evaluating areas of convergence, divergence and progression in this region can contribute to SC policy debates and development worldwide.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation program iPSpine under grant agreement No. 825925.
Keywords: Ethics, Policy, Regulation

TOPIC: KIDNEY

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ALLOGENEIC BONE MARROW MESENCHYMAL STEM CELL-DERIVED EXOSOMES ALLEVIATE HYPOXIC ACUTE TUBULAR INJURY IN HUMAN PROXIMAL TUBULE ON A CHIP

Çam, Sefa Burak - *Department of Histology and Embryology, Hacettepe University Faculty of Medicine, Ankara, Turkey*
Çiftci Dede, Eda - *Department of Bioengineering, Hacettepe University Graduate School of Science and Engineering, Ankara, Turkey*
Gürbüz, Nazlıhan - *Department of Bioengineering, Hacettepe University Graduate School of Science and Engineering, Ankara, Turkey*
Altun, Bülent - *Department Of Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey*
Korkusuz, Petek - *Department of Histology and Embryology, Hacettepe University Faculty of Medicine, Ankara, Turkey*

Abstract: Mesenchymal stem cells (MSC) and MSC exosomes (MSC-Exos) are strong therapeutic candidates for ischemic acute kidney injury (AKI) but their safety and efficacy remain ambiguous. MSC-Exos gave contradicting outputs when applied to proximal tubule (PT) epithelial monolayers and murine surgical ischemia models to test their potential for ischemic AKI. We hypothesized that therapeutic potential of allogeneic human bone marrow mesenchymal stem cell exosomes (hBMMSC-Exos) may be assessed on microfluidic-based 3D culture platform to quantify functional effects of the hBMMSC-Exos on acute hypoxic tubular injury. In brief, hBMMSC-Exos were isolated with ultracentrifugation, characterized by TEM and BCA assay. Real-time impedance-based cell proliferation analysis (RTCA) determined the treatment window for the hBMMSC-Exos on HK-2 cells in hypoxic conditions. The acute hypoxic tubular injury was modelled on a microfluidic-based 3D culture platform under 1% O2 for 48 hours. The proliferation of PT cells was assessed with WST-1 assay. 20 kDa and 155 kDa probes were used to assess and quantify epithelial barrier integrity. hBMMSC-Exos were characterized with high protein content (3694 ± 439.2 µg/ml) by BCA and typical spherical vesicles with bilayer membrane under TEM with mean dimensions of 52.18 µm and 51.27 µm. We described the effective treatment window of hBMMSC-Exos on PT cells under normoxia as 26 hours after application according to the changes in ED50 in RTCA. ED50 of hBMMSC-Exos under hypoxia was detected as 172.582 mg/ml at 26th hour. hBMMSC-Exos alleviated cell proliferation after 24 hours ($p< 0.0001$) after hypoxic tubular injury. Barrier integrity assay revealed that hBMMSC-Exos ameliorated epithelial barrier integrity after injury for both 20 kDa ($p=0.0004$) and 155 kDa ($p< 0.0001$) dextran probes. In this study, the authors described a series of potency assays demonstrating real-time proliferative ED50 of allogeneic hBMMSC-Exos on PT on a chip, and validating the cellular alleviating effect of hBMMSC-Exos on an optimized 3D microfluidic platform. The real time platform presents a powerful tool for future precision medicine works with hBMMSC-Exos on AKI in terms of patient specific personalized efficacy and treatment window to ease the translation of the results obtained to nephrology clinic.

Funding Source: Hacettepe University Research Fund financially supported this work (TSA-2020-18383).
Keywords: BMMSC Exosomes, Proximal Tubule on a Chip, Acute Kidney Injury

TOPIC: NT - GENERAL

957

PLASMA TREATED AND BIO-FUNCTIONALIZED 3D POLYSTYRENE SCAFFOLDS AS HUMAN MESENCHYMAL STEM CELL EXPANSION PLATFORMS

Wong, Kuan Un - *School of Life and Environmental Sciences, The University of Sydney, New South Wales, Australia*
Zhang, Anyu - *School of Biomedical Engineering, The University of Sydney, Australia*
Akhavan, Behnam - *School of Biomedical Engineering, The University of Sydney, Australia*
Bilek, Marcela - *School of Biomedical Engineering, The University of Sydney, Australia*
Yeo, Giselle - *School of Life and Environmental Sciences, The Univeristy of Sydney, Australia*

Abstract: Mesenchymal stem cells (MSCs) hold great potential for regenerative medicine and tissue engineering applications due to their therapeutic potential. [1] However, due to the relatively rare natural occurrence of MSCs in vivo, long-term ex vivo expansion is necessary to achieve the high cell dosage required for cell therapies. Conventional planar (2D) expansion systems are costly, require constant renewal of soluble growth factors, and produce modest cell yields. Moreover, long-term expansion on 2D substrates impairs the phenotype, function and therapeutic value of MSCs. To address these issues, a cost-effective cell culture scaffold that better recapitulates the native MSC microenvironment, improves cell yield and maintains cell stemness is needed. We developed 3D-printed porous polystyrene scaffolds, which enhance cell yield by maximising the surface area-to-volume ratio, and which retain MSC function by mimicking the 3D architecture of the biological stem cell environment. We activated the surfaces of the 3D porous scaffolds using plasma immersion ion implantation (PIII) [2] to stably attach labile mitogenic agents, such as fibroblast growth factor 2 (FGF2), over the cell expansion period. We examined the stability of the surface-attached FGF2, and the proliferation and phenotype of seeded MSCs. Our results demonstrated that FGF2 proteins were covalently attached onto the scaffolds. Furthermore, the PIII-treated and FGF2 biofunctionalised scaffolds promoted MSC proliferation while maintaining MSC phenotype over 14 days. The increased cell yield, preserved cell stemness and reduced reagent usage associated with our scaffolds point to their utility as large-scale MSC expansion systems. Such biomimetic 3D expansion platforms will improve the translational feasibility of MSC clinical applications, and help re-

veal the multiplexed factors that govern stem cell fate and functionality.

Keywords: Stem cell expansion, plasma surface modification, Growth factor immobilisation

959

ARTIFICIAL INTELLIGENCE IN STEM CELL SCIENCE & REGENERATIVE MEDICINE: A MACHINE LEARNING-BASED POLICY APPROACH TO DEVELOPMENT, TRANSLATION, AND MANUFACTURE OF ADVANCED THERAPEUTIC PRODUCTS

Jakimo, Alan L. - *Principal, JakimoLaw, Woodbury, NY, USA*

Abstract: Worldwide growth of the cell and gene therapy infrastructure, based in large part on stem cell science and regenerative medicine ("SCSRM"), has generated, and will likely continue to generate, unprecedented volumes of data. Processing, organizing, curating, analyzing, and comprehending this data, and feeding back the resulting comprehension to support further scientific, medical, and health care endeavors will, without doubt, spur ongoing innovation in the SCSRM field. Many of these innovations will be particularly relevant to development, translation, regulatory approval, and manufacture of advanced therapeutic products. At the same time, the development and use of information technology tools commonly referred to as "artificial intelligence" ("AI"), "machine learning", and "deep learning", are evidencing substantial growth across many industries, including life science and health care. As a consequence of this growth, these AI-related terms have become a bit like the proverbial elephant, with each of its body parts under inspection by different investigators, in each case in the absence of eyesight. The machine learning study reported here provides both a quantitative and qualitative exploration of the intersection of SCSRM and AI designed to overcome the elephant description problem, and suggests policies for fostering continuing development and translation of advanced therapeutic candidates into readily and economically manufacturable products for broad-scale administration to patients.

Keywords: advanced therapeutic products, machine learning, development, translation, manufacture

POSTER SESSION III: ODD

3:00 PM – 4:00 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

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ADIPOSE-DERIVED STEM/STROMAL CELLS WITH HEPARIN-ENHANCED CELL MIGRATION AND ANTIFIBROTIC EFFECTS MITIGATE INDUCED PULMONARY FIBROSIS IN MICE

Saito, Takashi - *Department of Legal Medicine, Osaka Medical and Pharmaceutical University, Takatsuki-shi, Japan*
Kotani, Takuya - *Department of Internal Medicine IV, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
Matsuda, Shogo - *Department of Internal Medicine IV, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
Sato, Takako - *Department of Legal Medicine, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
Suzuka, Takayasu - *Department of Internal Medicine IV, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*
Takeuchi, Tohru - *Department of Internal Medicine IV, Osaka Medical and Pharmaceutical University, Takatsuki, Japan*

Abstract: Fibrosis is a general term for a group of diseases in which an organ is damaged and collagen fibers are overproduced during repair, resulting in the loss of elasticity and hardening. Interstitial lung disease (ILD) is a life-threatening pathological condition that causes respiratory failure and often presents as pulmonary fibrosis. Although it is treated using immunosuppressive and antifibrotic agents, their beneficial effects remain limited. Thus, development of new therapeutic strategies for lung fibrosis is crucial. Mesenchymal stem/stromal cells (MSCs) have multilineage differentiation potential. They have anti-inflammatory and fibrotic effects and the ability to modulate the immune response and modify the microenvironment at the site of engraftment. Adipose-derived MSCs (ASCs) are present in large numbers in the adipose tissue. ASCs are typically isolated via enzymatic digestion using collagenases. Heparin has been shown to have antifibrotic activity mediated by the cellular secretion of hepatocyte growth factor (HGF). The ability of low-molecular-weight heparin (LMWH) to secrete HGF is similar to that of normal heparin. It is secreted by mesenchymal cells and affects epithelial cell proliferation and morphology. The aim of this study was to confirm the therapeutic effect of LMWH-activated ASCs on ILD. Mouse ASCs (mASCs) were cultured in a LMWH-supplemented medium. Heparin and LMWH mediate several cytokines and growth factors related to cell migration and antifibrotic effects. LMWH significantly increased mASC number and enhanced migration and anti-inflammatory and antifibrotic effects. Mice with bleomycin-induced pulmonary fibrosis were intravenously administered LMWH-activated mASCs. The relative mRNA expression of inflammation-related genes in ILD lungs were significantly lower in the treatment group than in the pathological model group. LMWH-activated mASC administration reduced lung fibrosis. the addition of LMWH to the culture medium of ASCs regulated the antifibrotic, anti-inflammatory, and cell migration-related genes in ASCs. Intravenous administration of LMWH-activated ASCs to

mice with BLM-induced ILD reduced lung fibrosis. This stem cell-based therapy may be a new strategy for ILD treatment.

Funding Source: This work was supported by JSPS KAKENHI (Grant Number 21K16312).
Keywords: Adipose-derived mesenchymal stem cells, Low-molecular-weight heparin, Interstitial lung disease

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

605

RESCUE OF ISCHEMIC DISEASE BY TRANSPLANTING INTACT VESSEL STEM CELL/NICHE CELL CLUSTERS

Zhao, Liming - *Surgery, Stanford University, Palo Alto, CA, USA*
Lee, Andrew - *Cardiovascular Institute, Stanford University, Palo Alto, CA, USA*
Sasagawa, Koki - *Cardiovascular Institute, Stanford University, Palo Alto, CA, USA*
Wang, Yuting - *Surgery, Stanford University, Mountain View, CA, USA*
Stavitsky, Suzan - *Surgery, Stanford University, Palo Alto, CA, USA*
Weissman, Irving - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Palo Alto, CA, USA*
Longaker, Michael - *Surgery, Stanford University, Palo Alto, CA, USA*
Nguyen, Patricia - *Cardiovascular Institute, Stanford University, Palo Alto, CA, USA*
Chan, Charles - *Surgery, Stanford University, Palo Alto, CA, USA*

Abstract: Peripheral vascular disease remains a leading cause of vascular morbidity and mortality worldwide despite advances in medical and surgical therapy. Besides traditional approaches which only restore blood flow to pre-existing arteries, an alternative approach is to enhance the growth of new vessels, thereby, facilitating the physiological response to ischemia. Stem cell therapy holds promise as a minimally invasive approach to accelerate vessel regeneration and ischemia recovery. In our previous study, utilizing single cell sequencing, followed by prospective FACS isolation and transplantation, we identified two distinct vessel-forming stromal progenitor populations. Population 1 (P1) gives rise to stunted vessels and population 2 (P2) forms stunted vessels and fat in transplantation setting. Interestingly, co-transplantation of P1 and P2 cells is required to form functional vessels that improve perfusion in mouse hindlimb ischemia model. Built on our previous observations, we proposed that co-isolation and transplantation of P1/P2 with niche cells in their intact cluster will keep the native interaction and synergism to give rise to optimized vessel formation. In the present study, we established a new microfluidic based method (On-chip Sort) to isolate intact vessel stem cell/niche cell (VSC/NC) clusters and tested their ability to form vessels in vivo. We further tested transplanting of intact VSC/NC clusters in a mouse model of ischemic osteonecrosis. Laser Doppler imaging was used to measure blood flow of ischemic tissue, and micro-CT was used to assess changes of bone structure. Histologic analysis was also performed to show the bone cell death and bone structure change. We observed that isolated intact VSC/NC clusters gave rise to a higher frequency of host-derived intact vessel formation compared to VSC along group. In addition, transplanting of VSC/NC clusters resulted in restoration of blood flow around the cauterized vessel to supply the distal tissue. Finally, the bone cell viability and bone structure in ischemic area were remarkably improved in the VSC/NC clusters transplanting group. In conclusion, our findings describe a new approach to rescue ischemic osteonecrosis by co-isolation and transplantation of VSC/NC units as their intact organization.

Keywords: vessel stem cell, stem cell niche, ischemic disease

TOPIC: EPITHELIAL_GUT

607

HEPATOCYTE GROWTH FACTOR-INDUCED CELL PLASTICITY PROMOTES RECOVERY OF RADIATION-DAMAGED SALIVARY GLANDS

Choi, Dojin - *Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul*
Yoon, Yeo-Jun - *Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Lim, Jae-Yol - *Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*

Abstract: The salivary gland stem/progenitor cell depletion caused by radiotherapy to treat head and neck tumors leads to xerostomia. Many studies focused on stem cell enrichment to ameliorate radiation-induced xerostomia, but it remains elusive whether cell plasticity of stem/progenitor cell population exerts therapeutic effects on radiation-induced tissue damage. This study aimed to explore whether the hepatocyte growth factor (HGF) could induce cell plasticity of salivary stem/progenitor cells and contribute to the restoration of radiation-induced xerostomia using ex vivo organoid model and in vivo mice model. We detected alteration related to HGF/c-Met signaling at the protein and transcript level after irradiation in submandibular glands of mice. The organoid forming efficacy of irradiated salivary gland cells was recovered in HGF-containing culture conditions. We observed the up-regulation of epithelial-to-mesenchymal transition (N-Cadherin, Vim, Snai1), stemness (Sox2), basal cell (Krt5, Krt14) genes, and down-regulation of the luminal cell (Krt7) gene when HGF was added into organoid culture conditions after irradiation. HGF did not alter the Annexin V/PI staining plot and cleaved caspase-3 protein expression levels. We observed that the receptor of HGF and MAP kinases (Erk1/2 and p38) were phosphorylated in order when HGF was added into organoid culture conditions. The retroductal delivery of HGF into irradiated submandibular glands improved mice's body weight and salivary secretory function. Collectively, we demonstrate that HGF may have therapeutic potential by inducing cell plasticity of salivary stem/progenitor cells against radiation-induced damage.

Keywords: Hepatocyte growth factor, Cell-plasticity, Salivary gland organoid

609

EPITHELIO-STROMAL RELATIONSHIPS IN PHYSIOLOGY & IBD: OSTEOPONTIN A KEY FACTOR IN EPITHELIAL REGENERATION AND TUMOR INITIATION?

Hamel, Dimitri - *IRSD-INSERM U1220 & LAAS-CNRS, Toulouse, France*
Michel, Déborah - *IRSD, Toulouse, France*
Roy, Lauriane - *IRSD, Toulouse, France*
Lorrain, Victor - *IRSD, Toulouse, France*

Galey, Lucas - *IRSD, Toulouse, France*
Ferrand, Audrey - *IRSD, Toulouse, France*

Abstract: Inflammatory bowel disease (IBD) is characterized by inflammation of digestive tract mucosa. This pro-inflammatory environment leads to tissue damage affecting both the underlying stromal compartment and the epithelium. Under physiological conditions, the maintenance of homeostasis and epithelial renewal are provided by complex mechanisms controling the phenotype and capacities of the intestinal stem cells (ICS). Stromal cells, in particular the fibroblasts, actively participate in the establishment of a specific microenvironment (namely, the "niche") essential in this control. Our knowledge on whether fibroblast activation in IBD alters the phenotypic control of the epithelial cells remains limited. We thus investigated the impact of inflammatory fibroblasts on the epithelial compartment. After establishing human fibroblasts primary cultures from either normal (NAF) or inflammatory (IAF) colon areas of respectively healthy or IBD patients, we characterized the cytokines and growth factors secretion patterns of these two populations. We observed distinct secretory signatures and identified a factor specifically secreted by IAFs: the osteopontin (OPN). We investigated OPN effects on colon epithelial regeneration by treating colon organoids established from human normal colon mucosa (NORG) with different doses of recombinant OPN during 15 days. Based on HCS morphological analysis, we found that OPN increases NORG area and promotes immature structures enrichment, suggesting a direct effect on ISC and/or progenitor cells. To decipher the OPN-targeted cell population(s), we performed a transcriptomic analysis. We found that OPN upregulates the immatures markers expression, plasticity markers and OPN receptors (integrins & CD44), while repressing differentiation-associated markers. Further investigations are in progress to decipher whether OPN promotes normal epithelial cells phenotype switch into tumor-initiating cells.

Funding Source: Université de Toulouse Paul Sabatier III Région Occitanie Plan Cancer
Keywords: Fibroblasts, Stem cell niche, Regeneration

TOPIC: EPITHELIAL_LUNG

611

ENHANCED FATTY-ACID OXIDATION SLOWS THE CONTRIBUTION OF AT2 STEM CELLS IN THE LUNG REPAIR PROCESS AFTER A FIBROSIS-INDUCING INJURY

Hegab, Ahmed E. - *Division of Pulmonary Medicine and Medical Education center, Keio University and International University of Health and Welfare, Tokyo, Japan*
Kagawa, Shizuka - *Pulmonary Medicine, Keio University, Tokyo, Japan*
Koichi, Fukunaga - *Pulmonary Medicine, Keio University, Tokyo, Japan*
Ozaki, Mari - *Pulmonary Medicine, Keio University, Tokyo, Japan*

Abstract: The course of lung fibrosis development varies from patient to patient. The fibrosis process involves a combination of fibroblasts activation, accumulation of extracellular matrix, inflammation, and aberrant activation of epithelial cells. High-fat diet (HFD) was shown to induce lung inflammation, aberrant activation of stem cells, and lung mitochondria impairment. However, the effect of HFD on lung fibrosis is not yet identified. Groups of mice were fed standard diet (SD) or HFD. Then they were given a fibrosis-inducing lung injury with bleomycin, then examined for fibrosis severity, repair, and resolution at 3, 6, and 9 weeks after injury. At 3 weeks, no significant differences in inflammation and fibrosis severity were observed between SD- and HFD-fed mice. However, infiltration of alveolar type (AT)-2 cells and bronchioalveolar stem cells (BASCs) into the fibrotic areas (the start of repair) was impaired in HFD-fed mice. At 6 weeks, SD-fed mice showed near-complete resolution/repair of fibrosis and inflammation, while HFD-fed mice still showed residual fibrosis and inflammation. Infiltration of the fibrotic areas with AT2 cells was observed, but very few BASCs were detectable. At 9 weeks, mice from both groups showed complete resolution/repair of fibrosis and inflammation, indicating that HFD induced delayed, rather than failed, resolution of fibrosis and alveolar repair. To further confirm the direct role of enhanced fatty-acid oxidation (FAO) in delayed resolution/repair, we administered etomoxir, a FAO inhibitor, to HFD-fed mice for 3–6 weeks after bleomycin injury. Inhibition of FAO abolished the HFD-induced delay in alveolar repair and fibrosis resolution at both time points. After a fibrosis-inducing injury, HFD slows resolution of fibrosis/inflammation and delays alveolar repair by slowing the contribution of AT2 stem cells and abolishing the contribution of BASCs in the repair process. FAO activation appears to be involved in this delay mechanism; thus, inhibiting FAO may be useful in the treatment of lung injury and fibrosis.

Keywords: lung stem cells, lung fibrosis, high fat diet

TOPIC: EPITHELIAL GUT

613

LONG-TERM SALIVARY GLAND ORGANOID CULTURE ENABLING MAINTENANCE OF DISTINCT GLANDULAR PROPERTIES OF MURINE AND HUMAN MAJOR SALIVARY GLANDS

Yoon, Yeo-Jun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Kim, Donghyun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Lee, Soohyun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Hwang, Seungyeon - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Kim, Jisun - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Cho, Jae-Min - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Choi, Dojin - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*
Lim, Jae-Yol - *Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea*

Abstract: Maintaining diverse salivary gland cells in organoids remains problematic. We established long-term murine and human salivary gland organoid cultures based on neuregulin-1, retinoid, and a combination of FGFs. This Wnt3a-free culture decreased basal cell-rich round organoids but increased heterotypic morphology of organoids. Murine and human salivary gland organoids expressed genes and proteins of acinar, myoepithelial, and duct cells and exhibited glandular secretory functions when stimulated with neurotransmitters. Furthermore, each organoid from parotid, submandibular, and sublingual glands generated gland-specific mucin and enzymes. Single-cell RNA sequencing indicated that human salivary gland organoids contained heterogeneous cell types and replicated glandular diversity. Our proto-

col also enabled the generation of tumoroid cultures from benign and malignant salivary gland tumor types, in which tumor-specific gene signatures were well conserved. Our study will provide an experimental platform for exploring precision medicine in the era of tissue regeneration and anticancer treatment.

Keywords: salivary gland organoid, salivary gland tumoroid, single cell RNA-sequencing

TOPIC: EYE AND RETINA

615

HUMAN STEM CELL-DERIVED RETINAL GANGLION CELLS MIGRATE INTO THE GANGLION CELL LAYER IN VIVO PRIMARILY THROUGH MULTIPOLAR MIGRATION

Soucy, Jonathan R. - *Ophthalmology, Schepens Eye Research Institute of Mass. Eye & Ear, Boston, MA, USA*
Baranov, Petr - *Ophthalmology, Schepens Eye Research Institute of Mass. Eye & Ear, Boston, MA, USA*

Abstract: Glaucoma and other optic neuropathies lead to the permanent loss of retinal ganglion cells (RGCs). Cell transplantation has been proposed to restore RGCs, and one of the significant barriers to successful RGC integration into the existing retinal circuitry is cell migration towards their natural connecting points. During development, RGCs, like most early-born neurons, migrate via somal translocation (ST), but confocal traces in zebrafish demonstrate that RGCs migrate through multipolar (MP) migration if ST is inhibited. MP migration does not rely on the extension and attachment of neural processes to reach their final location and is the preferred migratory mode for late-born neurons that navigate through developed tissues. It is unknown if RGCs are capable of MP migration in mammals, and it is not clear if the same rules apply to donor RGC in the transplant setting. To study donor RGC migration in vivo, human RGCs, differentiated from Brn3b-tdTomato hESC in organoid cultures, were transplanted subretinally in mice. Stromal cell-derived factor-1 (SDF1) was delivered intravitreally to establish a chemokine gradient across the retina to direct migration. To assess the transplantation outcome, retinas were stained for donor and host RGCs three days after transplantation, and we quantified the position of each integrated donor RGC in a 3D reconstruction of the host retina with respect to the ganglion cell layer. Our results show that inhibiting MP migration by roscovitine significantly limits the percentage of donor RGCs that migrate to the GCL in response to SDF1 (No inhibition: 45 ± 15%; MP inhibition: 20 ± 3.7%), whereas inhibiting ST by CK666 does not affect donor RGC migration (ST inhibition: 47 ± 12%). The inhibition of migration did not affect survival, with approximately 2.5% (578 ± 707 cells) of donor RGCs detected in each neural retina (n = 5-8 mice per group). Each migration pattern was confirmed with an in vitro time-lapse study. Inhibiting ST with CK666 resulted in a significant decrease in RGC migration speed from 5.6 ± 2.2 to 4.7 ± 2.0 µm/s, whereas inhibiting MP migration with roscovitine decreased their speed to 3.2 ± 1.5 µm/s – demonstrating hESC-derived RGCs can migrate via both modalities. Together, these results show that MP migration is the primary mode of migration for surviving donor human RGCs in the retina.

Funding Source: This work was supported by HMS Ophthalmology, Iraty Award, NIH/NEI grant U24EY029893, P30EY003790, T32EY007145, and F32EY033211
Keywords: Migration, Transplantation, Retinal Organoid

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

617

INVESTIGATING THE TRANSCRIPTOMIC PROFILE OF STEM CELLS FROM GESTATIONAL TISSUES TO TRUMP CANCER AND RESTORE TISSUE HOMEOSTASIS

Corradetti, Bruna - *Nanomedicine Research, Houston Methodist Research Institute, Houston, TX, USA*
Vaiasicca, Salvatore - *DISCLIMO, Università Politecnica delle Marche, Ancona, Italy*
Melone, Gianmarco - *Center of NanoHealth, Swansea University Medical School, Swansea, UK*
James, David - *Center of NanoHealth, Swansea University Medical School, Swansea, UK*
Conlan, Robert - *Center of NanoHealth, Swansea University Medical School, Swansea, UK*
Francis, Lewis - *Center of NanoHealth, Swansea University Medical School, Swansea, UK*

Abstract: The environment created during embryogenesis contributes to controlling inflammation and reducing aberrations that drive structural malformations and tumorigenesis. Placenta tissues, including the chorionic villi (CV) and the amniotic fluid (AF) play a role in supporting organogenesis, delivering nutrition, and offering a natural immune response against infections. Mesenchymal stem cells (MSC) isolated from these sources represent a valuable resource for the development of therapeutics with the potential to modulate the immune system towards tissue homeostasis following injury, disease and cancer. In this study we evaluated MSCs from CV and AF in terms of their transcriptional profile, to identify specific signatures that could be exploited for the development of immunomodulatory stem cell-based therapeutics to counteract cancer initiation and progression and inhibit chronic inflammation. Next Generation Sequencing analysis revealed molecular signatures (mRNAs, miRNAs, lncRNAs) with the potential to inhibit biological processes involved in the etiology of aggressive cancers (ovarian, pancreatic, etc). With some of the inhibitory molecules being incorporated into extracellular vesicles, in vitro validation demonstrated that the media conditioned by CV- and AF-MSC (and purified exosomes) exert a cytotoxic effect on two human ovarian cancer cell lines (OVCAR and SKOV-3), which was not found on normal cells. In addition, we identified molecular signatures that regulate immune pathways at the basis of the impaired immune response caused by chronic disorders (including COVID-19-cytokine storm), including HIF-1, IL-17, Toll-like receptors, RAP1, TNF, WNT, PI3K-Akt and NF-kappa B signalling. We also identified molecules responsible for tissue protection and repair (VEGF, IL-1, TGF-β1, EGFR) and extracellular matrix re-organization. This study lays the foundation for the development of stem cell-based biomimetic strategies for precision medicine. The successful incorporation of such MSC-based therapies into the treatment regimen for chronic disorders (including cancer) has the potential to reduce the morbidity associated to cancer initiation and progression by inducing tissue homeostasis and specifically targeting cancer cells.

Funding Source: Dr. Corradetti is supported by Sêr Cymru II programme (Horizon 2020, MSCA COFUND scheme), the Welsh European Funding Office under the European Regional Development Fund (ERDF) and Houston Methodist Research Institute.

Keywords: placental stem cells, anti-tumor potential, chronic disorders

TOPIC: HEMATOPOIETIC SYSTEM

619

IDENTIFICATION OF PRE-MEP AND PRE-GMP SUBSETS: TWO NOVEL MYELOID PROGENITOR CELLS WITHIN THE BONE MARROW

Mack, Ryan M. - *Cancer Biology, Loyola University Chicago, Oak Park, IL, USA*
Joshi, Kanak - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Sellin, Mark - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Zhang, Lei - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Liu, Shanhui - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Wei, Wei - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Breslin, Peter - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*
Zhang, Jiwang - *Cancer Biology, Loyola University Chicago, Maywood, IL, USA*

Abstract: Elucidating the stepwise differentiation processes that leads from multipotent hematopoietic stem cells to mature effector cells is critical for understanding both normal and neoplastic hematopoiesis. Early studies suggested that common myeloid progenitors (CMPs) are oligo-lineage hematopoietic progenitors that produce all lineages of myeloid cells, through bi-lineage megakaryocyte-erythroid progenitors (MEPs) and granulocyte-monocyte progenitors (GMPs). This concept was challenged after isolation of lineage-restricted subsets within the CMP population over the past 15 years, including monocyte-dendritic progenitors, megakaryocyte progenitors, and erythroid progenitors based on expression of CD115/Flt3, CD41/CD150, and CD105/CD150, respectively. However, the remaining CMP population is highly heterogenic and further separation of functional subsets is required. By screening cell surface markers on CMPs, we have identified CD27 as a reliable marker to separate all megakaryocyte/erythrocyte-committed progenitors from granulocyte/monocyte-committed progenitors. In addition, we found that CD62L is only expressed on granulocyte/monocyte-committed progenitors. CD27 and CD62L co-staining can separate CMP into CD27 +CD62L +, CD27 +CD62L - and CD27 -CD62L - subsets. Functional, gene expression, and morphology study showed that CD27 +CD62L - cells are closely associated with GMPs, whereas CD27 -CD62L - cells are closely associated with MEPs. In vitro culture and in vivo transplantation functional studies demonstrated that 1) CD27 +CD62L + cells are pre-GMPs that give rise to FcGRII/III + GMPs and only produce granulocytes and monocytes; 2) CD27 -CD62L - cells are pre-MEPs that give rise to MEPs and primarily produce erythrocytes and megakaryocytes with minimal contribution to granulocytes and monocytes; 3) CD27 +CD62L - subset enriches cells with genuine CMP potential capable of producing GMPs, MEPs, and subsequent progeny. Taken together, we have identified two novel populations of committed progenitors that serve as intermediates between CMP-GMP and CMP-MEP commitment pathways. Identification of pre-GMPs and pre-MEPs fills in the gap between CMPs-GMPs and CMPs-MEPs, supporting the hierarchal relationship of myeloid lineage differentiation.

Keywords: hematopoiesis, myeloid progenitors, HSPC

621

EXTRACELLULAR VESICLES ISOLATED FROM COCL2 TREATED MESENCHYMAL STROMAL CELLS REGULATE THE FATE OF HEMATOPOIETIC STEM CELLS IN VITRO

Pendse, Shalmali S. - *Symbiosis Centre for Stem Cell Research, Symbiosis International University (Deemed University), Pune, India*
Vaidya, Anuradha - *Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Pune, India*
Kale, Vaijayanti - *Symbiosis Centre for Stem Cell Research, Symbiosis International (Deemed University), Pune, India*

Abstract: Mesenchymal stromal cells (MSCs) reside in the hypoxic bone marrow microenvironment and regulate the fate of hematopoietic stem cells (HSCs). Hence, culturing MSCs under low oxygen concentration increases their regenerative potential. Although treating MSCs with atmospheric hypoxia (1% O2) or compounds such as CoCl2 establishes hypoxic conditions in them, they display variation in their hematopoiesis-supportive ability. We have previously shown that HSCs co-cultured with MSCs treated with CoCl2 (CoCl2-MSCs) exhibit long-term repopulation potential both in vitro and in vivo. However, HSCs co-cultured with MSCs exposed to 1% O2 (Hypoxic-MSCs) fail to expand in vitro and also exhibit compromised engraftment ability in vivo. Since MSCs are known to exert their salutary effect via paracrine mechanism, we wanted to assess the effect of extracellular vesicles – microvesicles (MVs) and exosomes (Exo) isolated from CoCl2-MSCs (Co-MVs and Co-Exo) and Hypoxic-MSCs on the regulation on HSCs. The overall trend suggested that when cultured with HSCs, Exo promoted better expansion of HSCs ex vivo as compared to the MVs. However, MVs improved the migration potential of HSCs as compared to Exo. Furthermore, when co-cultured with Co-MVs, HSCs displayed a poor proliferation potential, although, phenotypic analysis underscored their long-term repopulating ability. Furthermore, the colony forming unit assay demonstrated that the HSCs cultured with Co-MVs did not promote the formation of colonies of progenitor cells, whereas, those cultured with co-Exo promoted the formation of highly committed granulocyte-macrophage progenitor colonies. This suggests that perhaps, the co-MVs are responsible for maintaining HSCs in a quiescent state in vitro. Altogether our data demonstrate that the CoCl2-MSCs impart HSC supportive ability via secretion of MVs having a salutary effect and could have clinical implications as cell-free biologics for maintaining transplantable HSCs ex vivo.

Funding Source: Symbiosis International (Deemed University), Pune, India.
Keywords: Mesenchymal stromal cells, Hematopoietic stem cells, Hypoxia

TOPIC: LIVER

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THE POTENTIAL ROLES OF LIVER STEM CELL-DERIVED EXTRACELLULAR VESICLES AS A THERAPEUTIC TOOL FOR ALCOHOL ASSOCIATED LIVER INJURY

Meng, Fanyin - *Internal Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
Slevin, Elise - *Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
Wan, Ying - *Department of Pathophysiology, Southwest Medical University, Luzhou, China*
Harrison, Kelly - *Department of Transplant Surgery, Baylor Scott & White Memorial Hospital, Temple, TX, USA*
Xu, Wenjuan - *Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
Ashok, Shetty - *Institute for Regenerative Medicine, Department of Molecular and Cellular Medicine, Texas A&M College of Medicine, College Station, TX, USA*
Wu, Chaodong - *Department of Nutrition and Food Science, Texas A&M University, College Station, TX, USA*
Klaunig, James - *Department of Environmental and Occupational Health, Indiana School of Public Health, Indiana University, Bloomington, IN, USA*

Abstract: Alcohol associated liver injury (ALI) is a major cause of liver disease worldwide, both on its own and as a co-factor in the progression of chronic liver disorders. ALI is a public health issue that has very few treatment options. Liver stem cells are important to liver repair and could help to modulate damage done by ALI. Extracellular vesicles (EVs) are released by all cells in order to communicate with surrounding cells. This study aims to evaluate the therapeutic effects of EVs derived from liver stem cells during ALI. EVs were isolated from cultured human liver stem cells (LSCEVs) and analyzed for their contents compared to EVs isolated from cultured human hepatocytes (HHs) with a microRNA PCR array. C3H/HeOuJ mice were treated with 5% EtOH for 8 weeks with a binge every 10th day. LSCEVs were injected via the lateral tail vein at week 5 and week 7. Total liver was analyzed for fibrosis, downstream targets of let-7, inflammation, ductular reaction, proliferation and the epithelial to mesenchymal transition (EMT) with qPCR. LSCEVs were found to contain elevated levels of let-7 compared to EVs derived from HHs. Treatment with LSCEVs reduced levels of fibrosis as well as levels of Lin28a and HMGA2 in EtOH-treated mouse cholangiocytes. In EtOH-treated mice, Lin28a levels were increased but treatment with LSCEVs reduced Lin28a levels to normal. In total liver, the proliferation marker Ki-67 was increased in ethanol-treated mice but decreased to normal levels in LSCEV-treated ethanol fed mice. Measurement of EMT marker S100A4 expression showed that ethanol-treated mice had increased levels of S100A4, but this was decreased in LSCEV-treated ethanol-fed mice. Inflammation and ductular reaction, measured by qPCR of TGF-ß1, IL-6 and CK-19, was reduced in LSCEV-treated ethanol fed mice compared to ethanol-treated mice. The inflammation, ductular reaction and subsequent proliferation, EMT and fibrosis as a result of ALI can be reduced by treatment with LSCEVs which contain let-7. Modulation of let-7-containining LSCEVs could be a potential therapeutic option to reduce liver damage as a result of human alcohol-associated liver disease.

Funding Source: The study was supported by the VA Merit award, NIDDK R01 grants, NIAAA R21 grants, and PSC Partners Seeking a Cure Award to Dr. Fanyin Meng; and a Nature Science foundation of China grant (No. 81873563) to Dr. Ying Wan.
Keywords: Liver Stem Cells, Extracellular vesicles, Alcohol-associated liver injury

TOPIC: MUSCULOSKELETAL

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SINGLE CELL MULTI-OMICS ANALYSIS OF REGENERATING SKELETAL MUSCLE REVEALS SELF-RENEWING SATELLITE CELLS MARKED BY TGFBR3 AND REGULATED BY SMAD4

Okafor, Arinze E. - *Cell Biology, Duke University, Durham, NC, USA*
Lin, Xin - *Cell Biology, Duke University School of Medicine, Durham, NC, USA*
Situ, Chenghao - *Division of Life Science, Center for Stem Cell Research, Center of Systems Biology and Human Health, Hong Kong University of Science & Technology, Clearwater Bay, Hong Kong*
Wei, Xiaolin - *Cell Biology, Duke University School of Medicine, Durham, NC, USA*
Xiang, Yu - *Cell Biology, Duke University School of Medicine, Durham, NC, USA*
Wu, Zhenguo - *Division of Life Science, Center for Stem Cell Research, Center of Systems Biology and Human Health, Hong Kong University of Science & Technology, Clearwater Bay, Hong Kong*
Diao, Yarui - *Cell Biology, Duke University School of Medicine, Durham, NC, USA*

Abstract: Skeletal muscle has remarkable regeneration capacity. Successful muscle regeneration requires the orchestrated activation, proliferation and differentiation of satellite cells (SC) that are normally quiescent. Meanwhile, a subset of SCs must undergo a self-renewal process to replenish the stem cell pool. The regulation of self-renewal and differentiation fate of SCs ensures robust muscle regeneration and homeostasis throughout life. However, the molecular identity of self-renewing SCs remains elusive. Here, through integrative analysis of time-resolved single cell ATAC-seq and single cell RNA-seq datasets, we define the trajectories of SCs undergoing self-renewal versus myogenic differentiation at transcriptome and epigenome levels. We identify TGFBR3 as a marker of SCs undergoing self-renewal process that is controlled by the transcription factor SMAD4. Taken together, our results not only provide a foundation for comprehensive analysis of gene regulatory programs of muscle regeneration, but also reveal the molecular identity and the transcriptional control mechanism key for SCs self renewal.

Keywords: muscle regeneration, stem cell self-renewal, multi-omics

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COMPLEMENT FACTOR C1Q AS AN IMMUNE MEDIATOR OF MUSCLE AGING

Kanugovi, Abhijnya - *Neurology, Stanford University, Santa Clara, CA, USA*
Guerrero, Gabriel - *Neurology, Stanford university, Mountain View, CA, USA*
Rando, Thomas - *Broad Stem Cell Research Center, UCLA, Los Angeles, CA, USA*

Abstract: Aging is associated with a decline in regenerative capacity, homeostasis, and function of tissues. Age-related changes in muscle are attributable to functional decline of muscle stem cells (MuSCs, also called as satellite cells). These effects are a result, at least in part, of changes in the local and systemic environment in which cells reside. Heterochronic parabiosis studies from our lab demonstrated that old cells when exposed to a younger systemic environment gained youthful functions and, conversely, younger cells when exposed to aged blood showed reduced functions. Further studies from our lab showed that the systemic factor causing MuSC aging is a Wnt- activating factor and that inhibition of Wnt signaling restored youthful properties in MuSCs. C1q, a circulatory complement factor secreted by macrophages, whose activity increases in blood with age, was shown to activate Wnt signaling, thus affecting skeletal muscle regeneration in a deleterious manner. C1q deficiency or inhibition was shown to reduce muscle fibrosis in aged mice and improved regeneration. However, the direct cellular targets of C1q in muscle were unexplored. In this study, we show that C1q mediates its deleterious effects on muscle regeneration by directly acting on MuSCs, delaying their activation in response to injury and promoting fibrogenic transdifferentiation. Since muscle resident mesenchymal progenitors called fibroadipogenic progenitors (FAPs) are known to play an important role in muscle fibrosis, we wanted to study the effect of C1q on FAPs. We show that C1q drives fibrogenic differentiation of FAPs, which may contribute to age-associated muscle fibrosis. Using scRNA sequencing analysis, we show that FAPs are the major source of C1s, a downstream effector of C1q whose proteolytic activity is required for activation of Wnt signaling pathway and the downstream fibrogenic effects. Using pharmacological inhibitors of C1s and a mouse model for FAP-specific C1s depletion, we aim to understand the paracrine and autocrine signaling mechanisms involved in C1q mediated cellular effects. Together, our studies will broaden the understanding of systemic effectors of cellular aging and open new avenues to non-canonical functions of complement factors.

Keywords: Complement factors, Aging, Muscle stem cells

TOPIC: NEURAL

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TRANSPLANTATION OF HUMAN OLIGODENDROGENIC NEURAL PROGENITOR CELLS FOR THE TREATMENT OF CERVICAL SPINAL CORD INJURY

Pieczonka, Katarzyna - *Institute of Medical Science, University of Toronto,, ON, Canada*
Nakashima, Hiroaki - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Nagoshi, Nahirito - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Yokota, Kazuya - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Hong, James - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Badner, Anna - *Institute of Medical Science, University of Toronto, ON, Canada*
Chio, Jonathon - *Institute of Medical Science, University of Toronto, ON, Canada*
Shibata, Shinsuke - *Electron Microscope Laboratory, Keio University School of Medicine, Tokyo, Japan*
Khazaei, Mohamad - *Krembil Research Institute, University Health Network, Toronto, ON, Canada*
Fehlings, Michael - *Department of Surgery, University Health Network, Toronto, ON, Canada*

Abstract: Spinal cord injury (SCI) results in the loss of myelinating oligodendrocytes, ultimately contributing to impaired neural communication. Neural progenitor cell (NPC) transplantation is an attractive approach to replace the neural cells that have been lost following SCI and to promote beneficial trophic effects. However, the injury microenvironment predominantly directs NPCs to differentiate into scar-forming astrocytes as opposed to neurons or oligodendrocytes. In order to promote oligodendrocyte differentiation, we aimed to generate human induced pluripotent stem cell (iPSC)-derived oligodendrogenically-biased NPCs (oNPCs) and to characterise the neuroregenerative role of the cells in cervical SCI. oNPCs were prepared from iPSC-NPCs by mimicking oligodendroglial developmental cues in vitro. To characterise the cells in vitro, RT-qPCR and immunocytochemistry were used. For in vivo assessments, a cervical SCI was induced in RNU immunodeficient rats followed by transplantation with either oNPCs or vehicle two weeks post SCI. Behavioural recovery was monitored weekly for ten weeks post SCI. Following sacrifice, histological methods were used to determine oNPC differentiation, remyelination, astrogliosis, endogenous cell survival and tissue preservation. We found that several genes involved in oligodendroglial lineage determination were upregulated in the oNPCs compared to unbiased NPCs, including OLIG1, OLIG2 and SOX10. Immunostaining showed that the oNPCs gave rise to an increased proportion of oligodendrocytes (O1+; 47.2 ± 5.1%) than neurons (Tuj1+; 29.8 ± 3.1%) or astrocytes (GFAP+; 23 ± 3.7%) in vitro, and similar results were seen in vivo. Immunohistochemical analyses demonstrated that oNPC transplantation enhanced MBP+ remyelination, reduced GFAP+ astrogliosis and promoted the survival of endogenous NeuN+ neurons. Tissue preservation was higher in the oNPC group, as demonstrated using LFB and H&E staining. We observed no increase in the proliferation of oNPCs during the study. Importantly, oNPC transplantation correlated with significantly better grip strength and CatWalk gait scores compared to vehicle ($p < 0.05$). Overall, this work suggests that oNPCs can promote remyelination and several other neuroregenerative effects which correlate with functional recovery post SCI.

Funding Source: Wings for Life Krembil Foundation
Keywords: Spinal cord injury, Neural progenitor cells, Remyelination

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INVESTIGATION OF THE FUNCTIONAL INTEGRATION AND EFFICACY OF HESC DERIVED GRAFTS IN A MODEL OF HUNTINGTONS DISEASE

Garcia Jareño, Patricia - *Neurosciences, Cardiff University, Cardiff, UK*
Edwards, Olivia - *Neurosciences, Cardiff University, Cardiff, UK*
Fjodorova, Marija - *Neurosciences, Cardiff University, Cardiff, UK*
Lelos, Mariah - *Neuroscience, Cardiff University, Cardiff, UK*
Li, Meng - *Neurosciences, Cardiff University, Cardiff, UK*
Rosser, Anne - *Neurosciences, Cardiff University, Cardiff, UK*

Abstract: Huntington's disease (HD) is an autosomal dominant genetic disorder in which striatal medium spiny neuron (MSN) degeneration is predominant and occurs early. HD is associated with progressive deterioration of movement, cognition and behaviour, leading to death over a 20-30 year period post diagnosis. Currently, there are no approved disease-modifying treatments and symptomatic treatments are limited, although there are a number of potential disease-modifiers currently in early stage clinical trials. Cell replacement therapy, in which striatal MSN progenitors are transplanted directly into the adult striatum, is in development as a potential therapeutic intervention for HD patients. Preclinical data, using foetal-derived and pluripotent stem cell (PSC)-derived donor cells and proof-of-concept clinical studies, using foetal-derived donor cells, suggest that striatal grafts containing mature MSNs can alleviate motor and cognitive deficits. It is hypothesized that functional recovery relies largely on reconstruction of the basal ganglia circuitry that is disrupted in the Huntington brain. Thus the aim of this project is to determine whether grafts of human embryonic stem cell (hESC)-derived MSN-like cells form functional synapses with host brain neurons. We address this by mapping efferent and afferent graft-brain projections using monosynaptic rabies technology. We have created a hESC line expressing the TVA receptor and demonstrate that MSN progenitors differentiated from this cell line result in surviving grafts containing mature MSNs, following transplantation into the quinolinic acid (QA)-lesioned rat striatum. Our preliminary data demonstrates that host-to-graft connectivity is established with appropriate brain regions, and further exploration of host-to-graft and graft-to-host connectivity is ongoing, both in the QA-lesion and in HD transgenic animal models. These data will be important in establishing the ability of hESC-derived MSNs grafts to reconstruct damaged neural circuitry, and to facilitate our understanding of the mechanisms underlying recovery following hESC-derived cell transplantation.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813851.
Keywords: Huntington, Connectivity, Modified Rabies Virus

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ESTABLISHMENT OF A VASCULARIZED ISLET ORGANOID MODEL FROM HUMAN PLURIPOTENT STEM CELLS

Nguyen-Ngoc, Kim-Vy - *Departments of Pediatrics and Cellular & Molecular Medicine, University of California, San Diego, CA, USA*
Jun, Yesl - *Departments of Pediatrics and Cellular & Molecular Medicine, University of California, San Diego, CA, USA*
Bender, Hugh - *Department of Molecular Biology and Biochemistry, University of California, Irvine, CA, USA*
Sai, Somesh - *Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Berlin, Germany*
Hatch, Christopher - *Department of Molecular Biology and Biochemistry, University of California, Irvine, CA, USA*
Zhu, Han - *Departments of Pediatrics and Cellular & Molecular Medicine, University of California, San Diego, CA, USA*
Hughes, Christopher - *Department of Molecular Biology and Biochemistry, University of California, Irvine, CA, USA*
Sander, Maike - *Departments of Pediatrics and Cellular & Molecular Medicine, University of California, San Diego, CA, USA*

Abstract: Blood vessels play a critical role in pancreatic islet health and function. Native islets are densely vascularized and their interaction with surrounding blood vessels is important for beta cell function. Defects in islet vasculature could be causal to diabetes by promoting beta cell loss and dysfunction. Therefore, vasculature ought to be an integral component of an in vitro cell model for studying islet physiology and diabetes pathobiology. Current culture models of human pluripotent stem cell-derived islets (SC-islets) fall short in that they lack a vascular component. Here, we report establishment of a vascularized islet organoid model comprised of SC-islets, human primary endothelial cells (ECs), and fibroblasts (FBs). To analyze the function of beta cells in the organoid in real-time, we generated a GCaMP6f-hPSC line, in which GFP signal serves as a reporter for Ca2+ influx, a proxy for insulin secretion. Comparison of beta cell Ca2+ signals of non-vascularized and vascularized SC-islets revealed more frequent and stronger Ca2+ influxes in response to high glucose and the GLP-1 analogue exendin-4 in vascularized SC-islets. To identify vasculature-derived signals beneficial to beta cell function, we predicted cell-cell communication networks based on single cell RNA-seq data from SC-islets, ECs, and FBs. Among the top candidates were integrin receptors that interact with extracellular matrix components. Consistently, a significantly higher number of beta cells were in contact with EC-derived basement membrane proteins (e.g., laminin α1 and collagen IV) and exhibited activation of integrin beta1. The analysis also revealed BMP4-BMPR2 as a potential ligand-receptor pair mediating crosstalk between ECs and beta cells. Indeed, BMP4 addition augmented beta cell Ca2+ signal and SC-islet insulin secretion in response to high glucose and exendin-4. Finally, to develop a system mimicking in vivo physiology, we integrated SC-islets into a microfluidic platform where the organoid is supported by a network of perfused human microvessels. Our vascularized SC-islet organoid model will enable studies of crosstalk between beta cells and vasculature under conditions similar to native islet microenvironment. The model will also serve as a platform to study disease mechanisms of diabetes and test therapeutics.

Funding Source: UG3DK122639 (HIRN), 3-PDF-2017-386-A-N (JDRF)

Keywords: Beta cell function, Islet vasculature, hPSC-derived islets

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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DIFFERENTIAL REGENERATIVE CAPACITY OF PLACENTA-DERIVED MESENCHYMAL STEM CELL FROM TWO DISTINCT MATERNAL AGES DUE TO IN VITRO AGING

Fu, Cindy F. - *Department of Research in Sexual and Reproductive Health, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama*
Solis, Mairim - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama*
Guerrero, Erika - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama*
Vega, Shantal - *Department of Research in Sexual and Reproductive Health, Gorgas Memorial Institute for Health Studies, Panama*

Abstract: Maternal age has been known to influence the regenerative capacity in several types of adult stem cells, including mesenchymal stem cells. We have recently reported that human placental derived mesenchymal stem cells (PDMSC) from mothers aged 22-35 years have greater proliferation, multipotent and pluripotent biomarker expression, and multilineage differentiation ability. However, the exact moment when PDMSCs multipotency decline in-vitro after a prolonged cell expansion is unknown. Thus, the aim of this study was to understand the replicative limit in PDMSCs of two distant donor age groups, 18-21 and 31-35 years old, cultured for 3 and 23 passages. Our results revealed a 2-times increase in the size of PDMSCs from both maternal age groups at passage 23, 31-35 yrs at P23 and 18-21 yrs at P23, when compared to their counterpart at passage 3. PDMSCs from donors 31-35 yrs at P3 showed higher cell proliferation than those from 18-21 yrs at P3, though, PDMSCs from 18-21 yrs at P23 overtook the proliferative capacity of PDMSCs from donors 31-35 yrs at P23. Quantification in the expression of multipotent biomarkers, CD105, CD90, and CD73, showed a higher expression in PDMSC from 31-35 yrs at P23 and 18-21 yrs P23 compared to those in passage 3. A 3-fold increase in the osteogenic, adipogenic and chondrogenic gene expression of OCN, ALBP, and ACAN, was observed in PDMSCs from 31-35 yrs at P3 compared to the other groups. However, a dramatic decrease in the expressions of these differentiation markers were observed in PDMSC from 31-35 at P23, and not in cells from the 18-21 yrs age group. No expression of P21 gene was detected by RT-qPCR, suggesting that no senescence was present in any of the age groups. The results from the present study indicate that although an increase in the proliferation and differentiation potential was observed in PDMSCs from maternal ages 31-35 yrs at P3, a more sustained capacity was present in PDMSCs from mothers aged 18-21 years old after several passages. These findings underscore the importance that in vitro aging plays in stem cells from different maternal ages during prolonged periods of cell culture for future treatments based on cell therapy.

Funding Source: The work was supported by reaserch grands 09-2018-ITE17-R1-001, PAAC-NI-2020-II-61, and the Sistema Nacional de Investigación from the Secretaria Nacional de Ciencia, Tecnología e Innovación.
Keywords: Mesenchymal Stem Cells, Multipotency, Aging

TOPIC: PLURIPOTENT STEM CELLS

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HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELLING OF OSTEOGENESIS IMPERFECTA

Petzendorfer, Ellen - *Institute of Women's Health, University College London, UK*
Corecelli, Michelangelo - *Institute of Women's Health, University College London, UK*
Van Dijk, Fleur - *North West Thames Regional Genetics Service, London North West Healthcare NHS Trust, London, UK*
David, Anna - *Institute of Women's Health, University College London, UK*
Guillot, Pascale - *Institute of Women's Health, University College London, Milton Keyens, UK*

Abstract: Osteogenesis imperfecta (OI) is a heterogeneous group of inherited bone dysplasias characterised by bone fragility and low bone mass leading to increased risk of fractures. There is currently no effective treatment or cure. Mesenchymal stem cells (MSCs) are multipotent stem cells which can be differentiated into osteoblasts and have immunomodulatory and anti-inflammatory properties. In mouse models of OI, MSC transplantation can reduce bone fragility. However, when expanded in vitro, MSC undergo replicative senescence and progressively lose their ability to differentiate and proliferate. This reduces their clinical applicability. MSCs derived from pluripotent stem cells (iMSCs) represent an alternative to primary MSCs. They have been classed as rejuvenated MSCs, with a similar morphology, immunophenotype and differentiation potential to primary MSCs. We aim to develop a system utilising iMSCs and CRISPR-Cas9 gene editing to counteract bone fragility in OI. Urine epithelial cells from patients with type I (mild) and type III (severe) OI were reprogrammed to iPSC using integrating lentiviral vectors for the Yamanaka factors. The presence of the disease-causing mutation was validated in the iPSC clones and the mutation was corrected using CRISPR/Cas9. The corrected cells were detected using digital droplet and isolated using sib selection. Uncorrected OI iPSCs, CRISPR/Cas9 corrected iPSCs, and iPSCs from healthy donors were differentiated into iMSCs via the mesoderm lineage. iMSCs derived from all cell lines expressed the MSC markers CD73, CD90 and CD105 and did not express the hematopoietic markers CD34 and CD45. The iMSCs were capable of trilineage differentiation into osteoblasts, adipocytes and chondrocytes confirmed by alizarin red, oil red O, and alcian blue staining, respectively. Successful differentiation of iMSCs down the osteoblast lineage from normal and OI patient-derived iPSC cell lines will allow for the generation of an in vitro model of OI and subsequently evaluate the effect of the OI-causative mutation and ability of CRISPR-Cas9 gene editing to restore osteoblast function.

Keywords: Induced mesenchymal stem cells, Disease modelling, Osteogenesis imperfecta

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

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CYTOKINES AND GROWTH FACTORS EXPRESSION IN DIFFERENT TISSUE-DERIVED MESENCHYMAL STROMAL CELLS CULTURED IN FETAL BOVINE SERUM AND HUMAN PLATELET LYSATE

Mathur, Akanksha - *Nitte University Centre for Stem Cell Research & Regenerative Medicine (NUCSReM) K. S. Hegde Medical Academy (KSHEMA), NITTE (Deemed to be University), Mangalore, India*
Shetty, Siddharth - *Department of Orthopaedics, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Babu, Santhosh - *Department of Orthopaedics, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Shetty, Jayaprakasha - *Nitte University Center for Stem Cell Research and Regenerative Medicine, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Rao, Shama - *Institute of Regenerative Medicine, COM-Molecular and Cellular Medicine, Health Science Center, Texas A&M University, TX, USA*
Shetty, Veena - *Nitte University Center for Stem Cell Research and Regenerative Medicine, K. S. Hegde Medical Academy, Nitte (Deemed to be UniversityMangalore, India*
Basavarajappa, Mohana Kumar - *Nitte University Center for Stem Cell Research and Regenerative Medicine, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*

Abstract: Mesenchymal stem/stromal cells (MSCs) have been known to possess regenerative properties along with low immunogenicity that may reduce transplantation rejections. In this view, the current study aimed to comparatively investigate the levels of immunoregulatory cytokines and growth factors in MSCs among three different tissue sources expanded in fetal bovine serum (FBS) and human platelet lysate (hPL). Cartilage and infrapatellar fat pad (IFP) samples from osteoarthritis patients (n=3) were collected and plastic adherent C-MSCs and IFP-MSCs were established. Bone marrow (BM) samples from avascular necrosis cases (n=3) were collected and MSCs were isolated by Ficoll paque gradient density separation. All MSCs were isolated and established in 10% FBS and 5% hPL supplemented media. To assess the immunoregulatory cytokines secretion, cell lysates were prepared and evaluated for interleukin (IL)-4, 10, 6, 12, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Furthermore, enzyme-linked immunosorbent assay (ELISA) was performed by collecting the culture media. qRT-PCR results showed that the cytokine secretion of hPL cultured C-MSCs and IFP-MSCs was higher to that of BM-MSCs. Presence of hPL appeared to have increased the IL-4, IL-10 and TGF-β1 levels, with a lower levels of pro-inflammatory markers, such as IL-12, IL-6, TNF-α and IFN-γ. Levels of growth factors, such as basic fibroblast-like growth factor (BFGF), TGF-β1, insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), hepatocyte-like growth factor (HGF) and stromal-derived factor-1 (SDF-1) in MSCs with both supplements were also measured by qRT-PCR. Interestingly, BFGF, TGF-β1, IGF-1, HGF and SDF-1 concentrations were higher in hPL supplemented media compared to FBS. Levels of VEGF were low in C-MSCs, due to their avascular source. In conclusion, our findings indicated that hPL could serve as an ideal alternative for clinical use as a xeno-free substitute. In addition, the higher levels of anti-inflammatory cytokines and growth factors in MSCs cultured with hPL would possess enhanced immunomodulatory and regenerative properties.

Funding Source: This work was supported by Nitte (Deemed to be University), Mangalore, India
Keywords: Human platelet lysate, Immunomodulation, Cytokine, Growth factors, Fetal bovine serum

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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ROLE OF ENDOTHELIAL-MACROPHAGE PROGENITOR CELLS AS AN OUTSIDE-IN DRIVER OF PLAQUE GROWTH AND STABILITY IN ATHEROSCLEROSIS

Hassanshahi, Mohammadhossein - *Adelaide Medical School, University of Adelaide, Australia*
Williamson, Anna - *Adelaide Medical School, University of Adelaide, Australia*
Abdollahi, Helia - *Adelaide Medical School, University of Adelaide, Australia*
Toledo-Flores, Deborah - *Adelaide Medical School, University of Adelaide, Australia*
Schwarz, Nisha - *Lifelong Health, South Australian Health and Medical Research Institute, Adelaide, Australia*
Liyanyage, Sanuri - *Adelaide Medical School, University of Adelaide, Australia*
Salagaras, Thalia - *Lifelong Health, South Australian Health and Medical Research Institute, Australia*
Chen, Yung-Chih - *Atherothrombosis and Vascular Biology, Baker Heart and Diabetes Institute, Melbourne, Australia*
Peter, Karlheinz - *Atherothrombosis and Vascular Biology, Baker Heart and Diabetes Institute, Melbourne, Australia*
Bursill, Christina - *Adelaide Medical School, University of Adelaide, , Australia*
Psaltis, Peter - *Adelaide Medical School, University of Adelaide, Australia*

Abstract: Arterial adventitial changes occur early during atherosclerosis, involving growth and infiltration of endothelial-lined vasa vasorum and expansion of macrophages. We recently identified embryonically derived, bipotent endothelial-macrophage (EndoMac) progenitor cells in the adventitia of healthy mouse aorta. Here we studied their fate and impact on the development and stability of atherosclerotic plaques. We first used Flt3Cre x RosamTmG x Apoe-/- mice fed high cholesterol diet (HCD) to induce atherosclerosis. Although most aortic macrophages were Flt3-cre+ (GFP+tdTom-), indicating their origins from bone marrow haematopoiesis, ~25% were locally derived Flt3-cre- (GFP-tdTom+). Flt3-cre- macrophages increased three-fold after 24 w of HCD and showed increased proliferative activity, which peaked earlier after 4 w of HCD. As a possible source for these locally derived macrophages, we observed up to six-fold expansion of Flt3-cre- aortic EndoMac progenitors, that also peaked after 4 w of HCD before returning to pre-atherosclerotic levels after 24 w. Separate studies in Apoe-/- mice showed similar increases in EndoMac progenitors after 2 and 4 w of HCD, before they diminished in number with more advanced atherosclerosis. This was accompanied by upregulation and then downregulation of

the self-renewal and angiogenic functions of EndoMac progenitors in vitro. To directly study their role in atherosclerosis, we injected progenitors from GFP donor aortas into the carotid artery adventitia of Apoe-/- mice that had undergone tandem stenosis surgery to induce unstable plaques. Six weeks later, we identified abundant donor-derived cells in both adventitia and plaque of recipient arteries, that had formed new endothelial cells (~60% of engrafted cells) and macrophages (~20%). Compared to vehicle control, injection of progenitors also resulted in significant increases in plaque lipid content, collagen deposition, macrophage infiltration, haemorrhage and adventitial vascularisation, with a trend for increased plaque area. These results show that early atherosclerosis is associated with expansion and proliferation of local EndoMac progenitors, which give rise to macrophages and endothelial cells in plaque and adventitia, that can accentuate development of unstable plaques.

Keywords: Atherosclerosis, Adventitia, Endothelial-macrophage progenitor cells

TOPIC: EPITHELIAL_SKIN

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PHYSIOLOGICAL CONSEQUENCES OF DEFECTIVE CELL DEATH IN HAIR REGENERATION

Mantellato, Giada - *Center for Molecular Medicine Cologne, University of Cologne, Germany*
Niemann, Catherin - *Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany*

Abstract: Hair follicles (HFs) of mammalian epidermis undergo cyclic bouts of regeneration throughout lifetime in order to continuously form new hairs. Stem cells (SCs) residing in the HF bulge and hair germ fuel the hair regeneration cycle that comprises three phases: (1) growth (anagen), where bulge and hair germ cells proliferate to generate the new hair shaft; (2) regression (catagen), where part of the HF keratinocytes undergo apoptosis and surviving cells move upwards to regenerate the hair germ; and (3) rest (telogen), where stem cells are characterized by quiescence. Skin is dramatically remodelled during catagen, which is a crucial phase for the re-establishment of the new SC niche. However, the role of cell death and the underlying cellular and molecular mechanisms are still not well understood. Here, we investigate the physiological consequences and the impact of defective apoptosis on hair regeneration. In particular, we aimed to interfere with HF regression by overexpressing the anti-apoptotic protein Bcl-2 in murine epidermis (Bcl-2EOE mice), thereby blocking the mitochondrial apoptosis pathway. We show that Bcl-2EOE mice display a block in cell death during catagen after HF morphogenesis. Furthermore, increased HF cell survival led to the formation of an enlarged HF bulge compartment, characterized by SC markers like SOX9 and Keratin 15, and a change in the architecture of the SC niche during the rest phase. As a consequence, the following hair regeneration cycle was impaired since mutant mice showed a delay in the anagen onset, due to a stall in the SC proliferation. Mechanistically, prolongation of the rest phase correlated with the upregulation of quiescence markers and a downregulation of activating signals. Intriguingly, dermal white adipose tissue was also affected by the epidermal cell death block, displaying impaired lipolysis during catagen and a delay in adipocyte expansion at anagen onset. Taken together, our data demonstrate that a proper regulation of cell death during the hair cycle is required for the establishment and function of the SC niche. Importantly, our model gives new insights into cell autonomous and non-autonomous mechanisms of SC-driven hair regeneration and tissue remodelling.

Keywords: hair follicle, cell death, Bcl-2

TOPIC: EYE AND RETINA

967

AN ANALYSIS OF VSX2.MCHERRY-LABELLED PROGENITOR CELLS IN HUMAN RETINAL ORGANOIDS

Pramod, Hema - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*
Cuevas, Elisa - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*
Rashidi, Hassan - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*
Leong, Yeh Chwan - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*
Patel, Aara - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*
Sowden, Jane - *Developmental Biology & Cancer, UCL Great Ormond Street Institute of Child Health, London, UK*

Abstract: Three dimensional retinal organoids self-organise in vitro in a process resembling retinal development. Retinal organoid cells have potential clinical application for the treatment of retinal dystrophy. Specification of multipotential retinal progenitor cells (RPC) from pluripotent stem cells is a critical step, as all retinal neurons, including photoreceptor cells, are generated by proliferating RPC. The visual systems homeobox 2 gene, VSX2 (also called CHX10), is specifically expressed in RPC and is known to be essential for retinal development as recessive VSX2 mutations cause microphthalmia (small eye). In this study we employed CRISPR/Cas9 genome editing to introduce an P2A-mCherry fluorescent reporter into the VSX2 locus of a human embryonic stem cell line. The VSX2.mCherry reporter enabled tracking of the production, proliferation and differentiation of VSX2-expressing RPCs in a retinal organoid differentiation model. VSX2.mCherry stem cells were differentiated for 28 weeks and evaluated by immunohistochemistry, flow cytometry and single cell transcriptome analysis. 3D organoids expressing VSX2.mCherry formed by week 5. At early stages the VSX2.mcherry reporter labelled RPCs, co-labelled with the proliferation marker Ki67. The proportion of VSX2.mcherry cells decreased over time as the organoids increased in size. Later stage organoids formed a characteristic outer nuclear layer of cone and rod cells displaying photoreceptor markers, whereas the VSX2-mCherry cells primarily resided in the inner layer and co-labelled with bipolar cell or Muller glial cell markers. VSX2.mCherry cell populations in early compared to late-stage organoids showed distinct cell surface marker profiles, identified by screening CD marker lyoplates. We show that VSX2.mcherry progenitor-like cells express Muller glial cell markers. The generation of a VSX2.mCherry reporter line that provides visualisation of human RPCs provides a tool for application in retinal tissue engineering and the study of retinal development and disease.

Funding Source: This work was supported by the Medical Research Council; National Institute for Health Research, NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Hospital Children's Charity.
Keywords: Retinal organoids, VSX2, Retinal Progenitor Cells

TOPIC: KIDNEY

969

SUPERIOR THERAPEUTIC ACTIVITY OF KU FACTOR-TREATED STEM CELL 3D SPHEROID-DERIVED EXTRACELLULAR VESICLES AGAINST INTERSTITIAL CYSTITIS

Cho, Ssang-Goo - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Song, Kwonwoo - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Lim, Kyung Min - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Kim, Sehee - *Department of Stem Cell and Regenerative Biotechnology, Molecular & Cellular Reprogramming Center (MCRC), and Incurable Disease Animal Model & Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Kim, Aram - *Department of Urology, Konkuk University Medical Center, Konkuk University School of Medicine, Konkuk University School of Medicine, Seoul, Korea*
Lee, Soobin - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Choi, Yujin - *Department of Stem Cell and Regenerative Biotechnology, Molecular & Cellular Reprogramming Center (MCRC), and Incurable Disease Animal Model & Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
An, Jongyub - *Department of Stem Cell and Regenerative Biotechnology, Molecular & Cellular Reprogramming Center (MCRC), and Incurable Disease Animal Model & Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Bong, Hanbit - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Jang, Soo Bin - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Jeon, Tak-Il - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Kang, Geun-Ho - *Department of Stem Cell and Regenerative Biotechnology, Molecular & Cellular Reprogramming Center (MCRC), and Incurable Disease Animal Model & Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Kim, Sejong - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Park, Hyojin - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Shin, Yeokyung - *Department of Stem Cell & Regenerative Biotechnology and Incurable Disease Animal Model and Stem Cell Institute (IDASI), Konkuk University, Seoul, Korea*
Lee, Jeong Ik - *Department of Veterinary Obstetrics and Theriogenology, College of Veterinary Medicine, and Regenerative Medicine Laboratory, Center for Stem Cell Research, Department of Biomedical Science and Technology, Institute of Biomedical Science and Technology, Konkuk University School of Medicine, Seoul, Korea*

Abstract: Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic disease characterized by incapacitating pelvic pain. MSC-derived extracellular vesicles (MSC-EVs) are considered key mediators of the paracrine action of MSCs and show better biological activities than the parent MSCs, especially in the bladder tissue, which may be unfavorable for MSC survival. Here, we produced MSC-EVs using advanced three-dimensional culture with exogenous KU factor (K-a3D-EVs). Treatment with K-a3D-EVs led to significantly enhanced wound healing and anti-inflammatory capacities. Moreover, submucosal layer injection of K-a3D-EVs in chronic IC/BPS animal model resulted in restoration of bladder function, superior anti-inflammatory activity, and recovery of damaged urothelium compared to MSCs. Interestingly, we detected increased cargo level involved with wound healing in K-a3D-EVs, which might be involved in the anti-inflammatory activity of these EVs. Taken together, we demonstrate the excellent immune-modulatory and regenerative abilities of K-a3D-EVs as observed by recovery from urothelial denudation and dysfunction, which could be a promising therapeutic strategy for IC/BPS.

Funding Source: This study was supported by a grant from the National Research Foundation (NRF) funded by the Korean Government (Ministry of Education, Science, and Technology) under Grant numbers 2019M3A9H1030682.
Keywords: Extracellular Vesicles, Interstitial Cystitis, Mesenchymal Stem Cell

TOPIC: NEURAL

971

METABOLIC CONDITIONING OF NEURAL STEM CELLS TO INCREASE THE THERAPEUTIC POTENTIAL OF THEIR SECRETOME

Solá, Susana - *Research Institute for Medicines, Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*
Roxo, Catarina - *Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*
Oliveira, Raúl - *Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*
Sá Santos, Sónia - *Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*
Rodrigues, Rui - *Research Institute of Molecular Medicine António Lobo Antunes (iMM), Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal*
Xapelli, Sara - *Research Institute of Molecular Medicine António Lobo Antunes (iMM), Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal*

Abstract: Evidence has shown that neural stem cells (NSCs), crucial for memory processes in the mature brain, are also pivotal to buffer depressive behaviour. Recently, it has become also clear that NSCs induce neuroregeneration by a paracrine mediated mechanism. However, the number of these cells dops sharply with aging and individual lifestyle, while the metabolism is key in regulating their activity. Here, we aimed to explore the metabolic signature of the most protective secretome-producing NSCs while also testing the influence of mitochondrial regulators on the regenerative properties of NSC secretome. Preconditioning of NSCs with either injured conditioned medium or mitochondrial boosters was performed. These cells and the secretome they produced were called boosted NSCs and boosted CM, respec-

tively. Our results showed that boosted CM was capable of reducing death of injured neuron-like differentiating NSCs. A deeper characterization of boosted NSCs showed that these cells are more proliferative, present increased levels of mitochondrial fragmentation, lipogenesis markers and NAD+/NADH, while having lower levels of ATP and mitochondrial DNA. Notably, besides abrogating cell death, the boosted CM was shown to increase ATP and NADH levels in recipient neuron-like differentiating cells. At last, we stimulated healthy NSCs with serum derived from depressed mice, using the animal model of unpredictable chronic mild stress (uCMS). Again, uCMS boostedCM was more efficient in preventing cell death of neuron-like differentiating cells, when compared with the secretome derived from NSC stimulated with serum derived from healthy mice. UCMS boosted NSCs also exhibited a more proliferative phenotype and increased mitochondrial fission. Collectively, our data showed that profound metabolic alterations should occur in NSCs exposed to external signals to trigger the production and delivery of a protective secretome to injured differentiating recipient cells.

Funding Source: Supported by grants PTDC/MED-NEU/29650/2017, UIDB/04138/2020 and UIDP/04138/2020 from Fundação para a Ciência e a Tecnologia, Lisbon, Portugal.
Keywords: Secretome, Metabolism, Neural stress

973

BONE MARROW MESENCHYMAL STEM CELLS REVERSE ISCHEMIA INDUCED NEURONAL DEGENERATION IN RATS

Ishaque, Aisha - *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*
Qazi, Rida -e- Maria - *PCMD, ICCBS,UoK, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*
Salim, Asmat - *PCMD, ICCBS,UoK, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*
Khan, Irfan - *PCMD, ICCBS,UoK, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan*
Simjee, Shabana Usman - *PCMD, ICCBS,UoK, PCMD, ICCBS, Karachi, Pakistan*
Hasan Adli, Durriyyah Sharifah - *Division of Biohealth Science, University of Malaya, Kuala Lumpur, Malaysia*

Abstract: Pathologies of central nervous system give rise to immense neuronal deterioration that worsen gradually and become challenging to treat. Among these, cerebral ischemia (stroke) is associated with neuronal degeneration, resulting in complete or partial loss of function of the affected body part. Mesenchymal stem cells (MSCs) can be used to treat neurodegenerative disorders. Preconditioning of MSCs can improve their survival, migration, homing and regenerative properties. Current study deals with preconditioning of bone marrow MSCs with alpha pinene (AP) for in vitro neuronal differentiation to examine their in vivo regenerative potential in rat model of cerebral ischemia. Differentiated cells were assessed for neuronal markers to confirm their identity. Rats were divided into normal control, ischemia induced control, normal MSC and AP treated MSC (AP-MSC) transplanted groups. Ischemia was induced in adult male rats by ligating the carotid artery. Normal or AP-MSCs were administered in their respective groups and brain tissues were harvested after one week of cell transplantation and assessed at histochemical (TTC staining), histopathological (tissue architecture) and transcriptional (inflammatory mediators profile) levels. TTC staining indicated the infarcted brain region in ischemia induced control group as compared to normal control, whereas normal and AP-MSC transplanted groups showed marked reduction in the infarcted area as compared to ischemia induced group. Transcriptional profile of ischemic rat brain showed marked upregulation of HIF-1a along with inflammatory mediators IL-1b, IL-6 and TNF-a compared to normal control, while both transplanted groups showed reduced expression of these mediators. Histological analysis of ischemic control group indicated neuronal damage, vascular edema, vacuolation and distortion in the tissue architecture as compared to normal control, whereas these damages were found to be reduced and reversed in the treated groups. The overall findings of the study indicate that bone marrow MSCs hold immense potential in their native as well as in preconditioned states to reverse the neuronal damage induced in response to ischemic injury. Therefore the current study provides a promising therapeutic approach against the CNS abnormalities due to neuronal loss.

Funding Source: This study was partially supported by the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education, Malaysia (FP120-2020).
Keywords: Cerebral Ischemia, Neuro-pathologies, Alpha Pinene

TOPIC: PLURIPOTENT STEM CELLS

975

IMPLEMENTATION OF A FREEZE DRYING PROCESS FOR HYDROGEL-BASED SCAFFOLDS FOR CULTIVATION OF PLURIPOTENT STEM CELLS

Balsters, Johnn-Majd - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering, Wuerzburg, Germany*
Gepp, Michael - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*
Neubauer, Julia - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*
Zimmermann, Heiko - *Fraunhofer Project Center for Stem Cell Process Engineering, Fraunhofer Institute for Biomedical Engineering (IBMT), Wuerzburg, Germany*

Abstract: Biomaterials are gaining in importance in tissue engineering applications. They are needed to generate customized scaffolds for medical and pharmaceutical purposes. One purpose is the expansion of (human) induced pluripotent stem cells (iPSCs). Since the discovery of iPSCs and the possibility to differentiate them into any type of somatic cell, a new approach was created for personalized drug screening, disease modeling and cytotoxicity testing. With this new technology, patient-specific iPSCs can be generated from e.g. a skin biopsy to, virtually, each specialized cell type of human body. Functional growth surfaces for the production of high cell numbers (>108 cells) are still required to address the unmet need for pre-clinical applications such as 3D-bioprinting. Ultra-high viscous (UHV) alginates are a class of biocompatible biomaterials extracted from brown algae that is commonly used as a scaffold. Alginate can be used as a hydrogel-based thin scaffolds as well as spherical microcarriers. In both cases the mechanical properties, the porosity and the surface chemistry are modifiable. However, until now, there is

no possibility to store protein coated UHV-alginate scaffolds in a long-time period. Therefore, it is important to establish a reliable and scalable process that enables long-term storage for protein-coated alginate scaffolds. In this work, a freeze drying approach for Matrigel™-coated alginate microcarriers will be presented as a promising technique to overcome the current limitations using trehalose as a lyoprotective agent. After freeze drying the microcarriers were rehydrated and the morphology analyzed. The structure/shape of the microcarriers were well preserved and mechanically stable. Furthermore, human iPSCs were cultivated on the reconstituted microcarriers to validate the biological functionality (adhesion, proliferation, pluripotency). According to our data, the freeze drying approach for protein-coated alginate scaffolds is compatible with state-of-the-art hiPSC workflows and will contribute to the production of high quality hiPSC-derived cells.

Funding Source: This work was supported by the Bavarian Ministry of Economic Affairs, Regional Development and Energy.
Keywords: Human induced pluripotent stem cells, Freeze Drying, Alginate

TOPIC: MUSCULOSKELETAL

979

DIRECTED DIFFERENTIATION OF HUMAN IPSCS INTO MESENCHYMAL LINEAGES BY OPTOGENETIC CONTROL OF TGF-β SIGNALING

Wu, Josephine - *Biomedical Engineering, Columbia University, New York, NY, USA*
Vunjak-Novakovic, Gordana - *Biomedical Engineering, Columbia University, New York, NY, USA*

Abstract: TGF-β signaling modulates key mesenchymal stem/stromal cell (MSC) functions. Despite numerous studies on TGF-β signaling in MSCs, the results are inconclusive, in part due to inherent batch variability, inconsistent culture stability, and lack of spatiotemporal control over soluble factors. Optogenetics can offer unprecedented precision and dynamic control of cell signaling using light. We report the development of an optogenetic human induced pluripotent stem cell (hiPSC) based system for TGF-β signaling and demonstrate its utility in driving TGF-β mediated mesenchymal differentiations. We generated stable hiPSC lines expressing optogenetic TGF-β receptors which maintained normal karyotype and pluripotency. Following differentiation into MSCs (optohiMSCs), cells continued to express the optogenetic receptors and met all standard criteria for MSC characterization. In 2-week smooth muscle and tenogenic differentiations from optohiMSC monolayers, gene and protein level expression of the respective markers (alpha smooth muscle actin, calponin-1; collagen I, scleraxis) were significantly increased in groups which had TGF-β signaling activated by either soluble factor or optogenetic stimulation, compared to groups which received neither. Optogenetic stimulation proved effective in 3D as well, as evidenced by global quantitative proteome analysis after 3-week chondrogenic differentiations, with samples from 2 independent experiments. Hierarchical clustering on a heat map of relative protein expression showed that in each experiment, groups which received optogenetic stimulation had protein expression profiles more similar to the soluble TGF-β treated positive control group than the untreated negative control group which received neither soluble factor nor optogenetic TGF-β stimulation. We demonstrate the utility of our hiPSC-based optogenetic system for driving TGF-β mediated mesenchymal cell differentiations into smooth muscle, tenogenic, and chondrogenic lineages. The collected data suggest that optogenetic regulation of TGF-β signaling can enable spatiotemporally precise control of hiPSC differentiation into mesenchymal lineages, towards more comprehensive understanding of the role of TGF-β in regulating MSC function.

Funding Source: We gratefully acknowledge funding support by the NIH (grants EB027062, AR078707) and NSF GRFP (grant 1644869).
Keywords: TGF-β signaling, optogenetics, mesenchymal

ISSCR Merit Award Recipient

TOPIC: NEURAL

981

OPTIMIZATION OF CRISPR EDITING WORKFLOW AND HOMOLOGOUS RECOMBINATION EDITING EFFICIENCY FOR INTRODUCING SINGLE NUCLEOTIDE POLYMORPHISM FOR DISEASE MODELING IN IPSC

Yuan, Shauna - *Neurology, University of Minnesota Medical School, Minneapolis, MN, USA*
Rose, Jamee-Grace - *Neurology, University of Minnesota, Twin Cities, Minneapolis, MN, USA*
Smedley, Garrett - *Neurology, University of Minnesota, Twin Cities, Minneapolis, MN, USA*

Abstract: Genome editing using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology is a powerful tool for studying biological functions, having shown more reliable editing efficiency than other genome editing methods such as ZFN (Zinc finger proteins) or TALEN (Transcription activator-like effector nucleases). Pairing CRISPR with induced pluripotent stem cells (iPSC) can provide a highly effective method for modeling disease etiologies. However, editing iPSCs has proven difficult, as assuring cell survival and achieving high editing efficiency continue to be major obstacles, with extra variables being introduced depending on the target site. CRISPR can induce apoptosis via p53 activation, leading to poor cell health or death. Mitigation of apoptosis can improve cell survival after CRISPR and thus increase editing efficiency. Here we present an optimized protocol to carry out point mutation in iPSCs, increasing the efficiency by inhibition of p53 and optimization of editing reagents, resulting in faster generation of isogenic lines using CRISPR. We tested our protocol on three separate genomic loci and in multiple cell lines. With addition of p53 inhibition, we observed 20 fold improvement in the homologous recombination rate. With further optimization using homologous recombination enhancer to aide with the homologous recombination process and CloneR to enhance cell survival, we observed additional improvement of homologous recombination rate by 39 fold or up to 65% without using drug selection. We generated single mutations into non-demented control iPSCs with risk variants in the EIF2AK3 (Eukaryotic Translation Initiation Factor 2 Alpha Kinase 3) which have been associated with increased risk for development of Progressive Supranuclear Palsy, a neurodegenerative disease. We estimate the protocol can produce clonal cell lines with genetic edits from start to finish in 8 weeks for a single mutation, which significantly reduces the overall time required. Our protocol utilizes commercially available reagents, and therefore can be easily applied in both academic

and private labs for generation of isogenic iPSC lines for disease modeling.

Keywords: gene editing, CRISPR, iPSC

TOPIC: LIVER

983

DEVELOPMENT OF SMALL MOLECULE FLUORESCENT PROBES FOR HUMAN LIVER STEM CELLS WITH HIGH DIFFERENTIATION CAPIBILITY INTO THE FUNCTIONAL HEPATOCYTE

Nourbakhsh, Nooshin - *Stem Cell and Regenerative Biology, GIS, Singapore, Singapore*
Koe, Chwee Tat - *Laboratory of Precision Disease Therapeutics, Genome Institute of Singapore, Singapore, Singapore*
Lee, Yong An - *Stem Cell and Regenerative Biology, Genome Institute of Singapore, Singapore, Singapore*
Lek Chee Chong, Jonathan - *Stem Cell and Regenerative Biology, Genome Institute of Singapore, Singapore, Singapore*
Lim, Yee Siang - *Laboratory of Precision Disease Therapeutics, Genome Institute of Singapore, Singapore, Singapore*
Wuestefeld, Torsten - *Stem Cell and Regenerative Biology, Genome Institute of Singapore, Singapore, Singapore*

Abstract: Chronic liver diseases are considered a worldwide health concern as they are mostly incurable and can increase the risk of liver cancer. Currently, the only possible treatment for patients is liver transplantation. However, considering the limitation of the transplantation, including the donor liver shortage and the aggressiveness of the surgery procedure, stem cell-mediated liver regeneration therapy has emerged as a potential alternative approach. While the in vitro culture of liver stem cells (LSCs) can be derived from patient liver tissue; however, it was observed that clonal lines have different propensity to differentiate into the hepatocyte lineage. Therefore, there has been a demanding requisite for a novel approach that may facilitate prior identification of LSCs with the high differentiation capability into hepatocyte lineage (HpLSCs) from the counterpart cells with low capacity (non-HpLSCs). To address this, we have performed a cell-based screening of in-house generated small-molecule fluorescent chemical compounds library with validated cell models as HpLSC and non-HpLSC lines, respectively. Our preliminary results have identified several candidate compounds that confer selective staining with the HpLSCs over the fibroblast feeder cells and non-HpLSCs model. The further study aims to focus on the validation of these candidate compounds using various assays followed by the selection of top-performing compounds. As the aim of this project is to find a probe that can apply to detect the LSCs with high differentiation capability into hepatocytes, this study will serve as valuable tools in the clinical application of PdLSCs as well as the biotech industry fields.

Funding Source: Supported by the Young Individual Research Grant (MOH-OFYIRG19may-0017).
Keywords: Liver regeneration, Liver stem cells, Bio-imaging probe

TOPIC: HEMATOPOIETIC SYSTEM

985

INHIBITION OF P38 MAPK SIGNALING IN MESENCHYMAL STROMAL CELLS GENERATES MICROVESICLES AND EXOSOMES THAT DIFFERENTIALLY REGULATE HEMATOPOIETIC STEM CELL FUNCTION

Budgude, Pallavi M. - *Symbiosis Center for Stem Cell Research, Symbiosis International University (Deemed University), Pune, India*
Kale, Vaijayanti - *Symbiosis Center for Stem Cell Research, Symbiosis International University, Pune, India*
Vaidya, Anuradha - *Symbiosis School of Biological Sciences, Symbiosis International University, Pune, India*

Abstract: Mesenchymal stromal cell-derived extracellular vesicles (MSC-EV) like microvesicles (MV) and exosomes (Exo) play a vital role in regulating hematopoietic stem cells (HSC). The regulation of HSC by MV and Exo is dependent on the signaling pathways active in the MSC. Earlier, we showed that inhibiting p38 MAPK signaling in murine MSC (primed MSC) rejuvenated them and enhanced their HSC-supportive ability. Here we examined whether EV isolated from primed MSC also display similar HSC-supportive properties. We found that although total EV (pEV) from the primed MSC did not exhibit enhanced HSC-supportive properties like their parent cells, MV (pMV) and Exo (pExo), when applied as separate fractions, boosted HSC proliferation. Phenotypic analyses showed that the HSC co-cultured with pMV expanded long-term repopulating HSC, whereas pExo promoted expansion of short-term repopulating HSC. Both pMV and pExo enhanced the colony-forming capacity of HSC maintaining their multi-lineage potential. Self-renewal genes such as Bmi1, Ezh2, Notch2 and Dnmt1 were significantly upregulated in the HSC co-cultured with pMV or pExo, indicating both fractions independently boosted HSC self-renewal potential. However, differentiation genes such as Pu.1, Il7r and Cebpa were dramatically downregulated in HSC co-cultured with pMV but markedly increased in HSC co-cultured with pExo. These results show that perhaps pMV boosts HSC self-renewal (symmetric division) while pExo promotes self-renewal and differentiation (asymmetric division). In vivo studies revealed that HSCs co-cultured with pMV or pExo showed improved homing and engraftment abilities. Moreover, pMV promoted HSC quiescence by upregulating transcription factor Egr1, cell cycle inhibitor p21 and autophagy genes Becn1, Lc3a, Lc3b and Atg7, thereby improving HSCs' retention in the bone marrow. In contrast, pExo stimulated HSCs' cycling by inhibiting the Egr1/p21 axis, thus facilitating their mobilization to peripheral blood. Overall, this study signifies how inhibiting p38 MAPK signaling in MSC gives rise to pMV and pExo, having differential effects on HSC cultured with them. This method of reprogramming MSC to yield tailored EVs could be used to develop ready-to-use cell-free biologics for ex vivo modulation of HSC to address specific hematological disorders.

Funding Source: This study was funded by the Department of Biotechnology (DBT), Ministry of Science & Technology, New Delhi, Government of India. Project (No.BT/PR23620/MED/31/368/2017).
Keywords: Hematopoietic stem cells, Mesenchymal stromal cells, Extracellular vesicles

TOPIC: EPITHELIAL SKIN

987

MESENCHYMAL STEM CELLS AND WOUND-RELEASED FACTORS AMELIORATE HEALING IN RAT MODEL OF CHRONIC WOUNDS

Salim, Asmat - *Stem Cell Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, Karachi, Pakistan*
Zaidi, Midhat - *Stem Cell Laboratory, PCMD, Karachi, Pakistan*
Jameel, Fatima - *Stem Cell Laboratory, PCMD, Karachi, Pakistan*
Aslam, Shazmeen - *Stem Cell Laboratory, PCMD, Karachi, Pakistan*
Khan, Irfan - *Stem Cell Laboratory, PCMD, Karachi, Pakistan*

Abstract: Chronicity of non-healing diabetic wounds is associated with dysregulated release of healing mediators and unorganized cellular response. Stem cells with their regenerative potential and capability to release paracrine factors, can promptly tackle these imbalances. However, in chronic wound milieu, their functionality is compromised. In the current approach, we aimed to augment healing in diabetic wounds by transplanting stem cells, conditioned and potentiated with factors released in the later phase of normal healing. Isolated bone marrow mesenchymal stem cells (BM-MSCs) were characterized by immunophenotyping. The safe concentration of wound extract isolated at different time points from normal and streptozotocin induced diabetic rats was determined by cytotoxicity assay. Healing potential of conditioned MSCs was studied in in-vitro scratch assay and later in in-vivo wound models by observing wound contraction, evaluating gene expression level and studying microarchitecture of wound sites by H&E and immunohistochemistry. Stem cells conditioned with least cytotoxic concentration (10 µg/mL) of day 9 normal wound proteins (N9) illustrated pronounced closure of wound margin in the scratch assay. Similarly, gene expression analysis showed high levels of inflammatory genes, and reduced expression of angiogenic and proliferative genes in the corresponding D9-MSCs. In-vivo studies complemented these findings as N9-MSC treated diabetic wounds showed significant wound size reduction. Expression levels of genes associated with accelerated healing was higher in N9-MSC treated diabetic wound. Likewise, histological investigation demonstrated that treatment with N9-MSCs recapitulated the epidermal and dermal architecture. Elevated expression of α-smooth muscle actin (α-SMA) also confirmed enhanced vascularization in N9-MSC treated diabetic wound. The study concludes that MSCs conditioned with wound induced paracrine factors released in later phases of normal healing reduced ulceration, restored skin integrity and ameliorated healing by unhindered transition of diabetic wounds from inflammatory phase towards proliferative and remodeling phase.

Funding Source: This study is supported by the HEC grant no. 4094
Keywords: Cell transplantation, Conditioned stem cells, Diabetic wounds

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

102

SINGLE CELL MULTIMODAL ANALYSES REVEAL DISRUPTED NEURAL CREST REGULATORY NETWORKS IN HEART DEVELOPMENT

Ranade, Sanjeev - *Institute of Cardiovascular Disease, The Gladstone Institutes, San Francisco, CA, USA*
Whalen, Sean - *Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA*
Zlatanova, Ivana - *Cardiovascular Research Institute, University of California, San Francisco, CA, USA*
Van Soldt, Benjamin - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Nishino, Tomohiro - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Pelonero, Angelo - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Ye, Lin - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Wallace, Langley - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Huang, Yu - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Przytycki, Pawel - *Institute of Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA*
Alexanian, Michael - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Padmanabhan, Arun - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Costa, Mauro - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Gifford, Casey - *Medicine, Stanford University, Palo Alto, CA, USA*
Black, Brian - *Cardiovascular Research Institute, University of California, San Francisco, CA, USA*
Pollard, Katie - *Institute of Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA*
Srivastava, Deepak - *Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Communication between myriad cell types during organ formation underlies proper morphogenesis. In cardiac development, reciprocal signaling between mesoderm progenitor and neural crest cells is essential and disruption leads to congenital heart malformations, the most common human birth defect; however, mechanistic interrogation of temporal gene networks and cis regulatory elements in this crosstalk is limited. Here, we integrated single cell chromatin accessibility and transcriptomics to define an unbiased and temporal epigenomic landscape of the embryonic mouse heart. We developed machine learning models to predict enhancers for cardiac and neural crest development. We then determined the consequences of dysregulated signaling caused by disruption of TBX1, a transcription factor that causes morphogenetic defects of the cardiac outflow tract in humans and functions non-cell autonomously in cardiac progenitors to direct pharyngeal neural crest differentiation. Loss of Tbx1 led to

broad closure of chromatin regions enriched in cardiac progenitor transcription factor motifs within a subset of mesodermal progenitors and correlated with diminished expression of numerous members of Fibroblast Growth Factor, Retinoic Acid, Notch and Semaphorin pathways. In affected progenitors, ectopic accessibility and expression of posterior second heart field factors suggested impaired axial patterning. In response, we identified epigenomic and transcriptional defects in a subset of cardiac neural crest cells indicating a failure of differentiation and corresponding with decreased expression of caudal pharyngeal arch Hox genes. This study provides a mechanistic framework for how disruptions in cell communication affect spatiotemporally dynamic regulatory networks in cardiogenesis.

Keywords: single cell multiomics, neural crest, heart development

TOPIC: EARLY EMBRYO

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SET PROTECTS FROM ABERRANT H3K4ME3 DEPOSITION DURING EARLY EMBRYONIC DEVELOPMENT

Lim, Patrick Siang Lin - *Genetics, The Hebrew University, Jerusalem, Israel*
Meshorer, Eran - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: The multifunctional histone chaperone, SET, is involved in many cellular processes, including inhibition of histone acetylation and as an inhibitor of PP2A. SET-KO is embryonic lethal in mouse and in several organisms, SET is required for normal neural tube formation. In a pluripotent state, SET exists mainly in one isoform, SETα, whereas its alternative isoform, SETβ, is expressed in differentiated cells. We previously analysed SET-KO ESCs, but since the SET-KO cells differentiate prematurely and transition out of a naïve pluripotent transcriptomic state, it was difficult to assess the role of the individual isoforms. To overcome this, we generated SET-KO cell lines capable of Dox-inducible addback of either SETα or SETβ and performed time-course RNA-seq experiments. In addition, using epitope tags, we carried out isoform-specific ChIP-seq analysis for both SET isoforms followed by ChIC-seq analysis for H3K4me3 in the SET-KO cells. While the SET-KO ESCs exhibit slower proliferation and upregulation of several endoderm-specific genes, our time-course RNA-seq analyses identified isoform-specific functions for SETα-regulating genes in the early embryo and SETβ-regulating genes later in development. In addition, our ChIP-seq datasets uncovered SET binding at genes regulated by the H3K4 demethylase, KDM5B. In SET-KO ESCs, H3K4me3 levels are elevated and are more widespread. Overall, our results suggest a role for SET in the protection from aberrant H3K4me3 deposition during early embryonic development.

Funding Source: European Union's Horizon-2020 Marie Sk1odowska Curie EpiSyStem ITN Network (765966).
Keywords: pluripotency, SET, ESCs

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EFFECT OF CCL2 ON IN VITRO MATURATION OF PORCINE OOCYTES

Kim, So Hee - *Veterinary Medicine, Chungbuk National University, Cheongju, Korea*
Hyun, Sang-Hwan - *Veterinary medicine, Chungbuk National University, Cheongju, Korea*

Abstract: Chemokines play an important role in regulating the complex immune system at the maternal-fetal interface during pregnancy. Chemokine (C-C motif) ligand 2 (CCL2) is also a key periovulatory gene in the cumulus-oocyte complexes (COCs). In this study, we confirmed the concentration of CCL2 in porcine follicle fluid (pFF) by follicle size and identified CCL2 gene expression in porcine oocytes, cumulus cells (CCs), and granulosa cells (GCs) as well as its effects on the COCs during in vitro maturation (IVM). pFF was obtained from different follicle sizes to perform the ELISA method. The concentration of CCL2 in porcine follicular fluid was significantly higher as the size of the ovarian follicle increases. RT-PCR and qRT-PCR was also performed to identify the expression level of CCL2 in porcine follicular cells (Oocytes, CCs, GCs) before and after IVM. We divided two groups for RT-PCR and qRT-PCR : One group was treated with hormones during the first 22 hours of IVM and the other group was sampled immediately after aspiration of pFF. As a result, the CCL2 mRNA was expressed in GCs before and after IVM. However, CCL2 mRNA expression in oocytes and CCs appeared only after IVM. In the qRT-PCR results, the mRNA expression levels of CCL2 were significantly higher in the group that underwent IVM in all porcine follicular cells. During IVM, CCL2 was added to the maturation media (TCM199-PVA) at concentrations of 0 (control), 1, 10, and 100 ng/mL for each group. After IVM, the 100 ng/mL CCL2-supplemented group (91.6 ± 3.6%) showed a significantly higher ($p < 0.05$) metaphase II rate compared to the control (81.8 ± 1.8%). All CCL2-supplemented groups showed a significant ($p < 0.05$) increase of intracellular GSH levels compared with control. On the other hand, the intracellular ROS levels in all of CCL2-treated oocytes were significantly decreased ($p < 0.05$). These results indicate that CCL2 has a good effect on nuclear maturation and cytoplasmic maturation on porcine oocytes.

Funding Source: This work was supported by grants from the "NRF funded by the Korean Government (2017K1A4A3014959, 2020R1A2C2008276)" and "IPET in Food, Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.
Keywords: CCL2, in vitro maturation, porcine oocyte

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ALTERNATE PROMOTER REGIONS IN SOX17 ARE DIFFERENTIALLY REGULATED DURING ENDODERMAL AND VASCULAR ENDOTHELIAL DEVELOPMENT

Trinh, Linh T. - *Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA*
Osipovich, Anna - *Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, USA*
Sampson, Leesa - *Vanderbilt Center for Stem Cell Biology, Vanderbilt University, Nashville, TN, USA*
Wong, Jonathan - *College of Art and Science, Vanderbilt University, Nashville, TN, USA*
Wright, Christopher - *Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA*

Magnuson, Mark - *Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, USA*

Abstract: Sox17 gene expression is essential for both endothelial and endodermal cell differentiation. To better understand the genetic basis for expression of multiple Sox17 mRNA forms we identified and performed CRISPR/Cas9 mutagenesis of two evolutionarily conserved promoter regions (CRs). Deletion of the upstream and endothelial cell-specific CR1 caused only a modest increase in lympho-vasculogenesis via reduced Notch signaling downstream of SOX17. In contrast, deletion of the downstream CR2 region, which functions in both endothelial and endodermal cells, impairs both vascular and endodermal development causing death by embryonic day 12.5. Analysis of 3D chromatin looping, transcription factor binding, histone modification and chromatin accessibility data at the Sox17 locus and surrounding region further supports the differential regulation during development of the two promoters.

Keywords: Sox17, endoderm, vascular endothelium

TOPIC: GERMLINE

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TRIM28-MEDIATED REPRESSION OF ENDOGENOUS RETROVIRUSES SAFEGUARDS PRIMORDIAL GERM CELL DIFFERENTIATION

Di Russo, Jonathan - *Molecular Biology Institute, University of California, Los Angeles, , CA, USA*
Wang, Allison - *Molecular, Cell and Developmental Biology, University of California, Los Angeles (UCLA), CA, USA*
Tao, Yu - *Molecular, Cell and Developmental Biology, University of California, Los Angeles (UCLA), CA, USA*
Xiang, Xinyu - *Zhejiang University-University of Edinburgh Institute (ZJU-UoE Institute), Zhejiang University School of Medicine, International Campus, Haining, China*
Liu, Wanlu - *Zhejiang University-University of Edinburgh Institute (ZJU-UoE Institute), Zhejiang University School of Medicine, International Campus, Haining, China*
Clark, Amander - *Molecular, Cell and Developmental Biology, University of California, Los Angeles (UCLA), CA, USA*

Abstract: Mammalian Primordial Germ Cells (PGCs) undergo two fate-restriction events: specification, after which PGCs enter latent pluripotency, and determination, during which PGCs exit latent pluripotency and differentiate. In mouse pluripotent stem cells, Endogenous Retroviruses (ERVs) are increasingly appreciated as robust sources of regulatory elements, augmenting enhancer and promoter repertoires and harboring binding sites for core pluripotency factors, including NANOG, SOX2 and OCT4. As mouse PGCs (mPGCs) also express the core pluripotency network following specification, we set out to understand the contribution of ERVs to maintaining latent pluripotency in mPGCs and how this potential role is balanced against the threat posed by young, transposition-competent ERVs to the integrity of the germline genome. We hypothesize this balance is orchestrated in part by Tripartite-Motif containing 28 (TRIM28, aka KAP1). In mouse ESCs, TRIM28 represses young, transposition-competent ERVs, enabling ESC survival and self-renewal. To parse the contribution of TRIM28 to ERV regulation in mPGCs in vivo, we employed a PGC-specific conditional TRIM28 knockout model. We show via RNA-seq that loss of TRIM28 drives significant derepression of Class II ERVs including IAP-, RLTR- and MMERV-family elements as early as E11.5. This is accompanied by a significant reduction in mPGC number by E12.5, concomitant with mPGC determination. However, unlike loss of DNMT1 or PRC2 activity in mPGCs, TRIM28 mutant mPGCs do not differentiate precociously. Interestingly, TRIM28 knockout mPGCs do not exhibit evidence of extensive DNA damage or apoptosis via γH2AX and cPARP immunofluorescence, respectively. Instead, the reduction in mPGC number may be driven by observed differences in cell-cycle progression. We also observe transient downregulation of DAZL in the absence of TRIM28, suggesting that TRIM28-mediated regulation of ERVs is necessary for timely mPGC entry into determination. Collectively, these data show that TRIM28-mediated ERV repression is a critical guardrail ensuring mPGCs are competent to undergo determination.

Funding Source: UCLA Molecular Biology Institute Whitcome Fellowship, R01HD058047
Keywords: TRIM28/KAP1, Transposable Elements, Determination

TOPIC: HEMATOPOIETIC SYSTEM

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ELUCIDATING A QUALITY ASSURANCE MECHANISM FOR STEM CELLS

Pessoa Rodrigues, Cecilia - *Harvard Stem Cell & Regenerative Biology Department, Harvard University, Cambridge, MA, USA*
Wattrus, Samuel - *Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA, USA*
Rubin, Sara - *Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Medical School, Boston Children's Hospital, Boston, MA, USA*
Baron, Chloé - *Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Medical School, Boston Children's Hospital, Boston, MA, USA*
Zon, Leonard - *Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Medical School/Harvard Stem Cell & Regenerative Biology Department, Boston Children's Hospital/Harvard University, Boston, MA, USA*

Abstract: In many adult tissues, the maintenance of organ function depends on stem cells. Although these are required for tissue repair, it is unknown which signals are quality assured during their development. Our lab has previously identified macrophage-HSPC interactions during embryogenesis which quality assures the nascent stem cell pool. Macrophages either fully engulf stem cells or partially eat them. In the latter situation, the stem cell goes on to divide. This is mediated by the "eat me" signal calreticulin (CALR) on the HSPC surface. Nonetheless, the signals triggering CALR expression on the HSPC surface mediating their removal or amplification remain unknown. Single-cell RNAseq analysis of the adult zebrafish marrow revealed a continuum of calr levels among the HSPC expression states, which correlated with the FoxO signaling, a pathway known to respond to reactive oxygen species (ROS). Probing surface CALR in human hematopoietic cell lines and zebrafish embryos, ROS scavengers lower surface CALR expression and macrophage interactions, and foxO CRISPR targeted embryos showed ROS accumulation associated with high surface CALR levels and decreased HSPC numbers. To systematically evaluate pathways triggering surface CALR, a panel of 1200 FDA-approved bioactive small molecules in human

cells were screened. Surface CALR expression was assessed by imaging a CALR-antibody coupled with a fluorophore and by a SPLIT-TURBO ID construct targeting the association of CHD2 (Cadherin 2), a membrane protein, and CALR. 93 out of 1200 compounds screened increased surface CALR with a robust dosage response. Chemical annotation further supported that ROS+ drugs were associated with FOXO1A and oxidative stress, while the ROS- drugs were associated with G protein-coupled receptor signaling and cellular calcium ion homeostasis. In vivo ROS- drugs induced macrophage-stem cell interaction and grooming behavior, while ROS+ drugs enhanced the macrophage-stem cell interaction, but not grooming. Collectively, our work has identified ROS as a signal that upregulates surface CALR and promotes macrophage-stem cell interactions, safeguarding the development of stem cells that are stressed or damaged.

Funding Source: National Institute of Health grant 5T32HL007574-40 (CPR)
Keywords: Stem cell quality control, Cellular interaction, Cellular stress

TOPIC: KIDNEY

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THE TRANSCRIPTIONAL LANDSCAPE OF KIDNEY ORGANOID DEVELOPMENT PROVIDES INSIGHT FOR OPTIMISATION OF ORGANOID MODEL DEVELOPMENT

Wilson, Sean B. - *Cell Biology, Murdoch Children's Research Institute, Fitzroy, Australia*
Howden, Sara - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Muskovic, Walter - *The Kinghorn Cancer Centre, Garvan-Weizmann Centre for Cellular Genomics, Darlinghurst, Australia*
Vanslambrouck, Jessica - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Murphy, Angela - *The Kinghorn Cancer Centre, Garvan-Weizmann Centre for Cellular Genomics, Darlinghurst, Australia*
Powell, Joseph - *The Kinghorn Cancer Centre, Garvan-Weizmann Centre of Cellular Genomics, Darlinghurst, Australia*
Little, Melissa - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*

Abstract: Stem cell derived kidney organoids are complex tissues containing epithelial and mesenchymal populations reminiscent of in vivo kidneys. However, organoids have deficiencies including structural immaturity beyond an early trimester 2 equivalence and populations of non-renal cell types arising during differentiation. For the promise of organoids to be realised for personalised medicine and clinically relevant translation, these deficiencies need to be addressed. Through existing knowledge of in vivo kidney development, we generated organoids with targeted perturbations to our existing protocol to seek conditions under which we can optimise mesoderm specification and downstream kidney organoid development. For all conditions we collected samples at multiple key timepoints, generating a comprehensive single-cell dataset of organoid differentiation. This comprehensive transcriptional map of kidney organoid differentiation across time provides a platform for advanced investigation of kidney organoid developmental dynamics. The map identifies the heterogeneity that arises across organoid differentiation while highlighting conserved gene expression profiles that drive kidney organoid development. Recent studies have shown that the presence of appropriate stromal cells is crucial to the development of advanced nephron networks in kidneys, both in vivo and in vitro. However, the in vitro stromal populations that arise do not faithfully represent those in vivo, while the generation of unwanted off-target cell types may further impair appropriate nephron development. Our screen identified conditions that limited the differentiation of off-target populations by improving the maintenance of a nephrogenic identity. Investigation of stromal development literature suggested in vivo stromal cells originate from the paraxial, and not intermediate, mesoderm. In agreement with this, our dataset confirmed conditions in which populations expressing both paraxial mesoderm and stromal progenitor markers were present. Enriching for these cells will enable their addition to organoid differentiation cultures, potentially reproducing the in vivo environment required for nephron maturation and further advances in kidney organoid development.

Funding Source: Australian Research Council (SR1101002: Stem Cells Australia, DP190101705, DP180101405) and National Institutes of Health (UH3DK107344)
Keywords: Kidney organoid, Directed differentiation, Cell atlas

TOPIC: MUSCULOSKELETAL

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DISTINCT GENE REGULATORY NETWORKS IDENTIFIED ACROSS HUMAN SKELETAL MUSCLE DEVELOPMENT GUIDE IN VITRO MATURATION OF MUSCLE PROGENITOR TO STEM CELL STATES

Chien, Peggie J. - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Xi, Haibin - *Genetic Engineering, Poseida Therapeutics, Inc., San Diego, CA, USA*
Sparta, Breanne - *Integrative Biology and Physiology, University of California, Los Angeles, CA, USA*
Romero, Matthew - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Saleh, Kholoud - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*
Deeds, Eric - *Integrative Biology and Physiology, University of California, Los Angeles, CA, USA*
Pyle, April - *Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA*

Abstract: Skeletal muscle progenitor cells (SMPCs) and satellite cells (SCs) participate in developmental myogenesis and postnatal muscle regeneration, respectively. Differentiating human pluripotent stem cells (hPSCs) into healthy, regenerative SCs is valuable for developing cell therapies for muscular dystrophies. However, current hPSC directed myogenic differentiation protocols result in immature embryonic/fetal-like SMPCs, and it is not known how to mature them into SCs. One maturation approach we are evaluating is manipulating the epigenome to mediate the transcriptional processes that regulate the SMPC and SC states. To understand how myogenic development is transcriptionally regulated, we performed single cell ATAC sequencing (scATAC-seq) to evaluate differential chromatin accessibility between human SMPCs and SCs. We identified differences in accessible transcription factor (TF) binding motif enrichment and predicted TF binding. We also performed integrative analysis between scATAC-seq and single cell RNA sequencing data previously acquired in our lab to evaluate correlation between accessible

TF binding motif enrichment and TF expression. Collectively, we identified unique sets of TFs that differentially regulate embryonic SMPCs, fetal SMPCs, and postnatal SCs. In particular, the TFs of the nuclear factor I family may be key regulators in driving myogenic development. These findings are being used to manipulate transcriptional activity in hPSC-derived SMPCs to generate more regenerative states that ultimately can be used in treatments for muscle wasting diseases.

Funding Source: NIH/NIAMS NRSA Individual Predoctoral Fellowship (F31 AR078640), CDMD at UCLA Azrieli Graduate Student Award, BSCRC at UCLA Predoctoral Fellowship, NIH T32 Training Grant (T32 AR065972)
Keywords: muscle development, transcription factors, satellite cells

TOPIC: NEURAL

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THE ROLE OF ADNP IN TRANSCRIPTIONAL REGULATION OF HUMAN NEURAL STEM CELLS

Pelicano de Almeida, Mariana - *Cell Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Meert, Lize - *Cell Biology, Erasmus MC, Rotterdam, Netherlands*
Dekker, Mike - *Cell Biology, Erasmus MC, Rotterdam, Netherlands*
Nosowad, Karol - *Cell Biology, Erasmus MC, Rotterdam, Netherlands Antilles*
Poot, Raymond - *Cell Biology, Erasmus MC, Rotterdam, Netherlands*

Abstract: Activity-Dependent Neuroprotective Protein (ADNP) is one of the genes most frequently mutated de novo in individuals with autism spectrum disorders (ASD). Ablation of ADNP in mice is embryonically lethal due to neural tube closure defects. In mouse embryonic stem cells (mESCs), ADNP forms a gene-regulatory complex together with the chromatin remodeler CHD4 and the heterochromatin protein HP1 (ChAHP complex). The ChAHP complex represses gene expression by establishing local heterochromatin domains and is required to prevent endodermal gene transcription in mESCs and for specification towards the neuronal lineage. ADNP is the DNA-binding component of the ChAHP complex and in its absence the complex can no longer assemble. Nevertheless, the exact mechanism of action of the ChAHP complex remains unknown. In order to better understand the role of ADNP in human transcriptional regulation, we determined its protein interaction network and its genomic occupancy in human neural stem cells (hNSCs). To that end, we applied CRISPR-Cas9 to endogenously tag the ADNP gene with a FLAG-V5 tag in hNSCs. Subsequently, we used the FLAG tag to purify ADNP and employed mass spectrometry to identify its interaction partners. We find the ChAHP complex, first established in mESCs, is also present in hNSCs. More interestingly, we uncover other repressor complexes as protein partners of ADNP, such as the LSD1 and G9a complexes. Accordingly, we show ADNP co-immunoprecipitates with endogenous LSD1 in hNSCs. To determine the genome binding profile of ADNP, we used the V5 tag to perform chromatin immunoprecipitation coupled to next generation sequencing (ChIP-seq). This revealed 18 204 sites that are significantly enriched for ADNP. Most peaks were observed in introns (41%) or in promoter regions (29%). The remaining peaks were located mostly in distal intergenic regions (27%). In addition, we performed ChIP-seq for LSD1 in hNSCs. We find ADNP and LSD1 overlap at a subset of genomic regions, which we are currently characterizing. Using CRISPR/Cas9, we generated ADNP KO hNSCs. We are now investigating how the absence of ADNP affects gene expression, local chromatin structure, the binding of its protein partner LSD1 to the genome and the epigenetic landscape of hNSCs.

Keywords: human neural stem cells, cell state maintenance, autism spectrum disorders

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COMBINATORIAL BARCODED TRANSCRIPTION FACTOR SCREENING FOR NEURONAL VARIETY

Liliom, Hanna L. - *Genetics and Development, Columbia University, New York, NY, USA*
Mazzara, Pietro - *Genetics and Development, Columbia University, New York, NY, USA*
Buckley, Tyler - *Genetics and Development, Columbia University, New York, NY, USA*
Yoney, Anna - *Genetics and Development, Columbia University, New York, NY, USA*
Baldwin, Kristin - *Genetics and Development, Columbia University, New York, NY, USA*

Abstract: Generation of a more diverse array of human neuronal subtypes in vitro than what has been achieved to date carries value both from a translational point of view and for basic research. First, it has been shown that various neuronal subtypes have differential vulnerability in case of several human-specific neurological diseases. Recreating these specific – sometimes rare – subtypes in vitro provides us with the opportunity to build more accurate cellular models for a given disorder, and so to gain a better understanding of disease mechanisms, enable more efficient screening for drugs and perhaps to even serve as the basis of cell replacement approaches to treatment. Second, recreating the diversity of native human neurons in a dish could help us understand fundamental questions about what determines and maintains neuronal identity. Direct reprogramming of human fibroblasts by transcription factor (TF) overexpression is a robust approach for generating human induced neurons. It has been well established that it is possible to recreate different neuronal subtypes depending on the factors used for conversion. The aim of our study was to find TF combinations that effectively and reproducibly induce specific neuronal subtypes in vitro. Human TFs enriched in adult brain or characterized by a high prenatal expression pattern were cloned into a library of inducible vectors with unique DNA barcodes, which were then packaged into lentivirus through a pooled approach. By combining our barcoded library with a newly developed cell line edited to express fluorescent tdTomato protein upon the induction of a synapse-associated gene, we were able to perform transcriptomic analysis on individual induced neurons and concomitantly determine the originally overexpressed TF combination. Our preliminary results show that diverse neuronal subtypes can be generated by a wide variety of TF combinations and indicate that pairings of basic helix loop helix and pit-oct-unc-family TFs are enriched among neuron-inducing combinations. Applying this combinatorial screening approach to different cellular systems may afford us insight into the mechanistic principles governing transcription factor-induced direct reprogramming.

Funding Source: Research was supported by NIA, DP1 AG055944 to K.K.B., BBRF Young Investigator Grant to P.M.
Keywords: transdifferentiation, neuron, transcription factor

TOPIC: PANCREAS

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IDENTIFYING CELL TYPE SPECIFIC 3UTR EQTLS DIRECTLY FROM SINGLE-CELL RNA SEQUENCING DATA

de Winter, Twan J. - *Department of Internal Medicine, Leiden University Medical Center, Alphen Aan Den Rijn, Netherlands*
Alemany, Anna - *Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Carlotti, Françoise - *Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*
De Koning, Eelco - *Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands*

Abstract: The 3' untranslated region (UTR) of mRNAs plays an important role in mRNA regulation. Some genetic variants can directly influence this regulation, and thereby gene expression, because they may create or destroy interaction-sites of RNA binding proteins or regulatory RNAs. These so-called expression quantitative trait loci (eQTLs) are of particular interest in development or disease since the effect on gene expression can differ when cells change identities. Large eQTL databases (e.g., GTEx) exist but have mainly been generated from bulk sequencing of whole organ or tissue samples. It has been shown, however, that many eQTLs are cell type or even subtype-specific. Especially organs with rare cell types suffer from this "bulk" approach. One example is the pancreas, a dual organ of mostly exocrine tissue, which contains only 1-2% of endocrine cells. It is therefore not suitable to query the existing databases for certain endocrine subtype-specific eQTLs. To beat this limitation, we developed a computational pipeline to identify 3'UTR eQTLs by simultaneously extracting donor-specific 3'UTR variants and cell type-specific gene expression directly from single-cell RNA-seq datasets. We merged four published scRNA-seq datasets of pancreatic islets to increase genetic variation and statistical power. Around 50.000 3'UTR genetic variants could be called in all donors. 12% of the variants were found to be eQTLs in one or more cell types. Interestingly, around 80% of all eQTLs were significant in only one cell type. However, there is a strong correlation between cell types and number of called cell type-specific eQTLs (Pearson's r= 0.96), suggesting that it is more difficult to call eQTLs with a weaker effect in low abundant cell types. Next, we plan to experimentally validate some of the eQTLs in isolated pancreatic cells. Besides primary tissues, our pipeline can also be used to identify eQTLs between progenitor states during development or in vitro differentiation.

Keywords: genetics, Expression quantitative trait loci (eQTLs), RNA-sequencing

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COMPARATIVE ANALYSIS OF THE TRANSCRIPTIONAL LANDSCAPE OF STEM CELL DERIVED ISLETS FROM DIFFERENT PROTOCOLS

Schmidt, Mason - *Medicine, Washington University, St. Louis, MO, USA*
Ishahak, Matthew - *Medicine, Washington University St. Louis, MO, USA*
Millman, Jeffrey - *Medicine, Washington University St. Louis, MO, USA*

Abstract: Diabetes mellitus is a major global health issue which affects more than 400 million people, and its prevalence continues to grow. Stem cell derived β-cells (SC-β) differentiated from human pluripotent stem cells have the potential to provide an unlimited cell source to treat diabetes by cell replacement therapy. Recently, a variety of derivation protocols to obtain functional SC-β cells for research and therapeutic means have been developed. However, the transcriptomic differences between cells derived from differing protocols is not fully understood. Thus, it is important to characterize the heterogenous cellular populations that arise from these distinct derivation protocols and their subsequent transcriptomic signatures. To this end, we performed comparative analysis of three published single-cell RNA-sequencing datasets that employ unique, but well-established, SC-islet derivation protocols. Integrated analysis of transcriptomes from cells fully derived from each protocol confirmed that relatively similar endocrine cell-types emerged from each protocol. These populations include pancreatic endocrine cell types such as β cells, α cells, and δ cells. Each of the SC-islet derivation protocols also gave rise to multiple off-target cell populations. One of these off-target populations were enterochromaffin (EC) cells, which are detrimental to SC-islet function. Lastly, four β-cell subclusters, with unique transcriptomic profiles, were identified and their emergence depended on the derivation protocol employed. One of these subclusters of β-cells had a higher expression of insulin (INS), while another β-cell subtype had a higher expression of polyhormonal markers glucagon (GCG) and somatostatin (SST). These findings will prove to be a valuable resource for mapping and comparing the transcriptional landscape that accompanies stem cell derived islets.

Keywords: Islet, single-cell, Beta

TOPIC: PLURIPOTENT STEM CELLS

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TRANSCRIPTIONAL, EPIGENETIC AND METABOLIC REGULATORY PATHWAYS IN THE FIRST DAYS OF DEVELOPMENT

Zylicz, Jan J. - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Tjalsma, S. J. D. - *Hubrecht Institute, Utrecht University, Utrecht, Netherlands*
Bousard, Aurelie - *Insitut Curie, Insitut Curie, Paris, France*
Bages Arnal, Sandra - *reNEW, University of Copenhagen, Denmark*
Sato, Yuko - *Tokyo Institute of Technology, , Yokohama, Japan*
Teixeira da Rocha, Simao - *Universidade de Lisboa, , Lisbon, Portugal*
Kimura, Hiroshi - *Tokyo Institute of Technology, Yokohama, Japan*
Heard, Edith - *EMBL, Heidelberg, Germany*

Abstract: Successful development and stem cell differentiation is driven by a complex sequence of transcriptional, epigenetic, and metabolic changes. These do not operate in isolation as they are functionally coupled to one-another. How such complex regulatory networks coordinate specific cell-state changes remain largely unknown. Here I will present how, by tracking the dynamics of chromatin changes, we uncovered their functional relevance in mediating transcriptional repression in stem cells. To this end we used mouse development and X chromosome inactivation as a model. Indeed, by tracking the depletion and deposition of chro-

matin marks at the inactivating X we were able to unravel not only the precise choreography of epigenetic changes but also that histone deacetylation promotes transcriptional shutdown of X-linked genes. Conversely, Polycomb-associated H3K27me3 and SUVH420-dependent H4K20me1 are dispensable for the initiation of gene silencing. These dynamic chromatin studies were able to disentangle the cause from consequence in epigenetic regulation. We are now applying similar methods to unravel the upstream metabolic regulators of chromatin changes. In this respect we are focusing at the time of implantation, when facultative heterochromatin is formed and dramatic metabolic changes take place. Of particular interest is how one can disentangle functionally intertwined regulatory pathways as well as address how specificity of epi-metabolic regulation could be achieved during stem cell differentiation.

Funding Source: This research is supported by: The Novo Nordisk Foundation reNEW grant number NNF21CC0073729, Danmarks Frie Forskingsfond grant number 0169-00031B and Lundbeck Fonden grant number R345-2020-1497.
Keywords: Epigenetics, X inactivation, Metabolism

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POLYCOMB REPRESSIVE COMPLEX 2 SHIELDS NAÏVE HUMAN PLURIPOTENT CELLS FROM TROPHECTODERM AND MESODERM DIFFERENTIATION

Lanner, Fredrik - *CLINTEC, Karolinska Institute, Stockholm, Sweden*
Baqué-Vidal, Laura - *CLINTEC, Karolinska Institutet, Flemingsberg, Sweden*
Elsässer, Simon - *MBB, Karolinska Institutet, Flemingsberg, Sweden*
Kumar, Banushree - *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*
Navarro, Carmen - *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*
Petropoulos, Sophie - *CLINTEC, Karolinska Institutet, Flemingsberg, Sweden*
Salazar Mantero, Angelo - *MBB, Karolinska Institutet, Stockholm, Sweden*
Schell, John - *CLINTEC, Karolinska Institutet, Flemingsberg, Sweden*
Weltner, Jere - *CLINTEC, Karolinska Institutet, Flemingsberg, Sweden*
Winblad, Nerges - *CLINTEC, Karolinska Institutet, Flemingsberg, Sweden*
Zhao, Cheng - *CLINTEC, Karolinska Instritutet, Flemingsberg, Sweden*

Abstract: The first lineage choice made in human embryo development separates trophectoderm from the inner cell mass, which proceeds to form the pluripotent epiblast and primitive endoderm. Naïve pluripotent stem cells are derived from the pluripotent epiblast of the blastocyst and offer possibilities to explore how lineage integrity is maintained. Here, we discover that Polycomb repressive complex 2 (PRC2) maintains naïve pluripotency and restricts an intrinsic capacity of pre-implantation pluripotent stem cells to give rise to trophectoderm and mesoderm lineages. Through quantitative epigenome profiling, we find that a broad gain of histone H3 lysine 27 trimethylation (H3K27me3) is a common feature of naïve pluripotency. We define a previously unappreciated, naïve-specific set of bivalent promoters, featuring PRC2-mediated H3K27me3 concomitant with H3K4me3. Naïve bivalency maintains key trophectoderm and mesoderm transcription factors in a transcriptionally poised state that is resolved to an active state upon depletion of H3K27me3 via inhibition of the enzymatic subunits of PRC2, EZH1/2. Through bulk and single-cell transcriptomics we define a key role for PRC2 in maintaining naïve pluripotency. Inhibition of PRC2 forces naïve hESC into an 'activated' state through which differentiation into either trophectoderm or mesoderm lineages is triggered. This trajectory is distinct from embryonic lineage specification out of the post-implantation pluripotent state, hence PRC2-mediated repression provides a highly adaptive mechanism to restrict lineage potential during early human development.

Keywords: POLYCOMB REPRESSIVE COMPLEX 2, Naive stem cells, Trophectoderm

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NMD IS REQUIRED FOR TIMELY CELL FATE TRANSITIONS BY FINE-TUNING GENE EXPRESSION AND REGULATING TRANSLATION

Huth, Michelle - *Department of Microbiology, Immunobiology and Genetics, University of Vienna, Austria*
Santini, Laura - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs Vienna, Austria*
Galimberti, Elena - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs Vienna, Austria*
Titz-Teixeira, Fabian - *Institute for Genetics, Faculty of Math. Nat. Sciences, CECAD Cologne, Germany*
Oberhuemer, Michael - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs Vienna, Austria*
Beyer, Andreas - *Institute for Genetics, Faculty of Math. Nat. Sciences, CECAD Cologne, Germany*
Leeb, Martin - *Department of Microbiology, Immunobiology and Genetics, Max Perutz Labs Vienna, Austria*

Abstract: Cell fate transitions depend on balanced rewiring of transcription and translation programmes to mediate ordered developmental progression. Components of the nonsense-mediated mRNA decay (NMD) pathway have been implicated in regulating embryonic stem cell (ESC) differentiation, but the exact mechanism is unclear. Here, we show that NMD controls expression levels of the translation initiation factor Eif4a2 and its premature termination codon encoding isoform (Eif4a2 PTC). NMD deficiency leads to translation of the truncated eIF4A2 PTC protein, which elicits increased mTORC1 activity and translation rates and causes differentiation delays in NMD factor deficient mouse ESCs. This establishes a previously unknown feedback loop between NMD and translation initiation. Furthermore, our results show a clear hierarchy in severity of target deregulation and differentiation phenotype between NMD effector KOs (Smg5 KO> Smg6 KO> Smg7 KO), which highlights heterodimer-independent functions for SMG5 and SMG7. Together, our findings expose an intricate link between mRNA homeostasis and mTORC1 activity that must be maintained for normal dynamics of cell state transitions.

Keywords: cell fate regulation, nonsense-mediated mRNA decay, pluripotency

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HUMAN IN VITRO PLURIPOTENCY IS A CYCLE OF TRANSCRIPTIONALLY AND EPIGENETICALLY DYNAMIC LINEAGE-BIASED STATES

Seo, Seungmae - *Pediatrics, Columbia University, New York, NY, USA*
Kim, Suel-Kee - *Developmental Biology, Lieber Institute for Brain Development, Baltimore, MD, USA*
Shin, JooHeon - *Data Science, Lieber Institute for Brain Development, Baltimore, MD, USA*
Burke, Emily - *Data Science, Lieber Institute for Brain Development, Baltimore, MD, USA*
Chenoweth, Josh - *Developmental Biology, Lieber Institute for Brain Development, Baltimore, MD, USA*
Colantuoni, Carlo - *Data Science, Lieber Institute for Brain Development, Baltimore, MD, USA*
McKay, Ronald - *Developmental Biology, Lieber Institute for Brain Development, Baltimore, MD, USA*

Abstract: Human pluripotent stem cells (hPSCs) provide an opportunity to interrogate critical aspects of embryonic development. To effectively utilize hPSCs as models of normally developing or diseased tissue, it is essential to understand how they maintain pluripotency, undergo fate commitment, and compare these in vitro events to normal human embryos. Here, we present RNA-seq and ChIP-seq data across passaging, self-renewal, and early differentiation to reveal unexpected dynamics within pluripotency that contribute to the cells' potential. Transcriptional signatures defined three states of pluripotency (early, intermediate, and late) where early state cells (0-1 days) are biased towards mesodermal differentiation and late state cells (4-6 days) are biased towards neuro-ectodermal differentiation. The early state is characterized by higher expression of pluripotency genes, including NANOG, and early mesodermal genes such as NODAL, LEFTY1, and LEFTY2. In addition, autophagy-related genes were highly expressed in this early state resulting in the highest level of autophagic flux. An mTOR inhibitor, rapamycin, induced autophagy and subsequently attenuated progression to the late state. Once the cells transitioned to the late state, they started expressing neuroectodermal genes (NPTX1, SOX21, and OTX2), and the cells treated with BMP and TGFb signaling inhibitors diverged from the self-renewal at this state. To define the epigenetic basis of this transcriptional dynamic, we interrogated histone modifications at the same time points and conditions. The early state cells showed abrupt global downregulation of H3K4me3, while H3K9me3 and H3K27me3 levels gradually decreased as the cells entered the late state. Interestingly, ChIP-seq revealed that H3K4me3 peaks were enriched in enhancers and heterochromatin in the early state and became more biased to TSSs in the late state. We demonstrate how temporal heterogeneity in pluripotency regulates lineage emergence by focusing on transcriptional change, autophagy, and histone modification. These data model a local landscape on top of "Waddington's hill," where pluripotent cells cycle through defined transcriptional and epigenetic states with different potential upon every passage.

Keywords: Pluripotency, Transcription, Histone modifications

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EVOLUTIONARY ORIGIN OF VERTEBRATE OCT4/POU5 FUNCTIONS IN SUPPORTING PLURIPOTENCY

Morganti, Elena - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Sukparangsi, Woranop - *Department of Biology, Burapha University, Chon Buri, Thailand*
Lowndes, Molly - *NNF Center for Stem Cell Medicine reNEW, University of Copenhagen, Denmark*
Mayeur, Hélène - *Biologie Intégrative des Organismes Marins, CNRS, Sorbonne Université, Banyuls sur Mer, France*
Weisser, Melanie - *NNF Center for Protein Research, University of Copenhagen, Denmark*
Mazan, Sylvie - *Biologie Intégrative des Organismes Marins, CNRS, Sorbonne Université, Banyuls sur Mer, France*
Brickman, Joshua - *NNF Center for Stem Cell Medicine reNEW, University of Copenhagen, Denmark*

Abstract: In eutherian embryos, pluripotency is maintained across developmental stages, from naive pluripotency in peri-implantation to primed pluripotency at gastrulation with Pou5f1/OCT4 being central throughout. Here, we seek to understand the emergence of pluripotent states across vertebrates by coupling evolutionary analysis of the Pou5 gene family with functional in vitro studies using mouse Embryonic Stem Cells (mESCs) and reprogramming. By tracing the origin of the Pou5 family back to early vertebrates, we identify a single Pou5 gene in cyclostomes (jawless vertebrates) and two paralogs, Pou5f1 and Pou5f3 in gnathostomes (jawed vertebrates), that originated from a gene duplication event early in the evolution of this lineage. To test the ability of POU5 proteins from different points in evolution to support different facets of pluripotency, we ask if they can replace OCT4. We find that in species containing both paralogs, these proteins have undergone functional specialization and support either a naive or primed-like state, while the single cyclostome POU5 is incapable of supporting either. To better understand why the cyclostome POU5 is unable to rescue pluripotency and how the differences in these proteins evolved, we modelled protein structures across several species. While we find good conservation in the DNA-binding regions of the POU domains, the linker and regions flanking it have more sequence variability and structurally, the linker shows the largest shift in 3D space compared to mouse OCT4. By swapping pieces of these regions with their homologs from a jawed vertebrate with POU5F1 activity, we can both rescue the position of the linker region in silico and ability to support naïve pluripotency, suggesting that the positioning of the linker has been a crucial target in the functional evolution of POU5 proteins and sub-functionalization in naïve and primed pluripotency. We conclude that gene duplication in this family allows diversification and, in species where a single POU5 has been retained (e.g. mouse and human), its function changes to support both states. While the specific changes associated with these activities are diverse, they converge on the positioning of key 3D structural motifs and correlate with modes of germ cell evolution.

Funding Source: NNF21CC0073729
Keywords: Pou5f1/Oct4, Pluripotency, Evolution

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ELUCIDATING THE MOLECULAR BASIS OF DEVELOPMENTAL COMPETENCE DURING HESC LINEAGE-SPECIFICATION

Pulecio, Julian - *Developmental Biology, MSKCC, New York, NY, USA*
Tayyebi, Zakieh - *Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medical College, New York, NY, USA*
Liu, Dingyu - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Rickert, Robert - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Kaplan, Samuel - *Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medical College, New York, NY, USA*
Wong, Wilfred - *Computational Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Shukla, Abhijit - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Luo, Renhe - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Murphy, Dylan - *Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medical College, New York, NY, USA*
Apostolou, Effie - *Sanford I Weill Department of Medicine, Weill Cornell Medical College, New York, NY, USA*
Leslie, Christina - *Computational Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Huangfu, Danwei - *Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Abstract: Current models suggest that chromatin regulatory regions (CRRs) become transcriptionally competent upon binding of pioneer transcription factors (TFs), which reshape the 3D organization of the chromatin around them and initiate the cascade of events required for gene activation. However, the molecular determinants that confer transcriptional competence only to a defined set of CRRs at specific developmental stages remains elusive. Likewise, it is not clear if the chromatin conformation at early embryonic stages determines the future competence of a regulatory region or if the contact points between regulatory elements and promoters are gradually established after pioneer TF binding. Here we study the CRRs of PDX1 (a pancreatic progenitor TF) as a model to study how transcriptional competence is established during development. To identify the CRRs able to promote PDX1 expression at the earliest developmental stage we performed CRISPR-a screens, using the SAM activation system as a targetable pioneer TF, and discovered four CRRs able to cause PDX1 expression in hESC. These early competent CRRs did not have any noticeable enrichment in chromatin accessibility or H3K27ac signal. To determine if there were additional PDX1 CRRs that will be utilized later in the cell-lineage specification program, we performed CRISPR-i screens during the differentiation from hESC to the pancreatic lineage and discovered four additional PDX1 enhancers. This suggests that the binding of a pioneer TF to a CRR does not necessarily cause its activation, and that additional factors must influence its ability to promote gene expression. To dissect this, we performed chromatin conformation assays at the ES stage and found that a higher number of contact points between an enhancer and the PDX1 gene body does not correlate with its transcriptional competence. In parallel, we devised an epigenetic compound screen at the ES stage and found that HDAC inhibitors significantly increase the ability of the competent CRRs to activate PDX1 expression at early stages, but do not induce competence de novo to the other set of enhancers. This effect has been validated on multiple enhancers of several developmental genes. We are currently investigating how HDAC chromatin binding at the ES stage influences the competence of candidate CRRs of multiple genes.

Keywords: Developmental Competence, CRISPR screens, Chromatin Conformation

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DERIVATION OF EQUINE PLURIPOTENT STEM CELLS AMENABLE TO PRIMORDIAL GERM CELL SPECIFICATION

Wei, Yulei - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Yu, Leqian - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*
Wu, Jun - *Molecular Biology, UT Southwestern Medical Center, Dallas, TX, USA*

Abstract: Establishment of embryonic stem cell (ESC) lines has been successful in rodent and primate, but still lack of study of large animals. Here, by simultaneously activating FGF, TGF-β and WNT pathways, we developed a method that enabled the derivation of ESCs from large animal horse blastocysts and transgene-free induced pluripotent stem cells (iPSCs) from horse fibroblasts, which could also be directly induced into PGC-like cells in vitro. PGC responsive PSCs are invaluable for studying large animal pluripotency and early PGC development, and our method may be broadly applicable for the derivation of analogous stem cells from other large animal species.

Keywords: Horse pluripotent stem cell, PGC-like cells, Interspecies Chimerism

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BRCA1 AND 53BP1 REGULATE REPROGRAMMING BY MEDIATING DNA REPAIR PATHWAY CHOICE AT REPLICATION-ASSOCIATED DOUBLE-STRAND BREAKS

Wang, Ning - *Stem Cell Initiative, Columbia University Medicine Center, New York, NY, USA*
Georgieva, Daniela - *Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University Irving Medical Center, New York, New York, NY, USA*
Taglialatela, Angelo - *Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA*
Reczek, Colleen - *Northwestern University Feinberg School of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA*
Horiguchi, Michiko - *Department of Pharmaceutics, Tokyo University of Science in Yamaguchi, Sanyo-Onoda City, Sanyo-Onoda City, Japan*
Ciccia, Alberto - *Department of Genetics and Development, Columbia University Irving Medical Center, New York, NY, USA*
Baer, Richard - *Department of Pathology & Cell Biology, Columbia University Irving Medical Center, New York, NY, USA*
Egli, Dieter - *Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University Irving Medical Center, New York, NY, USA*

Abstract: Reprogramming to pluripotency is associated with DNA damage, presenting a primary limitation to the genetic and functional integrity of the resulting iPS cells. Efficient reprogramming

requires the functions of the BRCA1 tumor suppressor, which promotes genome stability through homology-directed repair of double-strand DNA breaks (DSBs), protection of stalled replication forks and suppression of ssDNA gaps. Here, we leverage the physical and/or genetic interactions between BRCA1 as its associated repair proteins to ascertain the relevance of homology-directed repair (HDR), stalled fork protection (SFP) and replication gap suppression (RGS) in somatic cell reprogramming. Surprisingly, loss of SFP alone was inconsequential for the transition to pluripotency in cells that retained HDR function. Conversely, the restoration of HDR activity through inactivation of 53bp1 rescued reprogramming in Brca1 mutants, and 53bp1-mutant cells showed elevated HDR efficiency and reprogrammed more efficiently than wild type controls. When exposed to genotoxic agents that preferentially induce replication-associated one-ended DSBs, reprogramming of 53bp1-mutant cells remained efficient, but was impaired after ionizing radiation, which induces two-ended DSBs. Furthermore, cells bearing combined phosphoserine mutations that abrogate the interactions of Abraxas, Bach1 and Ctip with Brca1 display specific deficiencies in HDR, and impaired reprogramming efficiency. These results show that the efficient repair of replication-associated one-ended DSBs by homologous recombination is limiting to somatic cell reprogramming, and can be improved through the loss of 53BP1.

Keywords: BRCA1 and 53BP1, Reprogramming, DNA Repair Pathway

TOPIC: EARLY EMBRYO

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ROLE OF HISTONE CHAPERONE APLF IN CELLULAR TRANSITION DURING EARLY MAMMALIAN EMBRYO DEVELOPMENT

Varghese, Pallavi Chinnu - *Regenerative Biology Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India*
Rajam, Sruthy Manuraj - *Regenerative Biology Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India*
Mukherjee, Ananda - *Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India*
Dutta, Debasree - *Regenerative Biology Program, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India*

Abstract: Lineage transitions play a vital role in embryonic development. The distinct differentiation of the embryo into inner cell mass, giving rise to embryonic stem cells and trophectoderm (TE), giving rise to trophoblast stem cells (TSCs), is one of the crucial stages associated with the cellular transition. Previously, we reported that histone chaperone, Aprataxin and PNK-like factor (APLF) is a regulator of epithelial to mesenchymal transition (EMT) implicated in the generation of induced pluripotent stem cells and breast cancer metastasis. Our present study is to investigate the role of APLF in cellular transitions during early mammalian embryonic development. We observed a dynamic expression pattern of APLF in pre-and post-implantation mouse embryos and also through different days of TSCs differentiation. APLF expression was significantly enhanced in TE and lineages derived from TE, which contributes to the extraembryonic lineage. Functional studies by shRNA mediated knockdown (kd) of Aplf in mouse embryos induced the hatching of embryos in vitro at E3.75, with a significant increase in Cadherin1 (Cdh1) and Caudal-type homeobox 2 (Cdx2) expression, and a significant failure in implantation was observed in vivo in Aplf-kd embryos. Rescue experiments neutralized the effects of Aplf knockdown both in vitro and in vivo. For the mechanistic study, we used differentiated TSCs as our model. Differentiation of Aplf-kd TSCs resulted in reduced expression of mesenchymal marker Snail Family Transcriptional Repressor 2 (Snai2) and TEA Domain Transcription Factor 4 (Tead4), and gain in epithelial marker Cdh1 level. Trophoblast differentiation mediated by EMT is crucial for embryo implantation, which is impaired in Aplf-kd embryos. Further studies are being conducted to unravel the underlying mechanism. Further validation of APLF being important for implantation was demonstrated by its significant expression within cytotrophoblast and syncytiotrophoblast cells in the full-term human placenta, human choriocarcinoma cell line BeWo and trophoblast derived HTR-8/SVneo cells. Our findings suggest a novel role for APLF during the implantation and post-implantation development of mouse embryos

Funding Source: DBT-Cellular Transition (BT/PR15498/MED/12/716/2015), intramural funds from the institute aided by DBT. DST INSPIRE fellowships (IF170833 and IF180251), DBT-Ramalingaswami Fellowship (BT/RLF/Re-entry/03/2016).
Keywords: EMBRYONIC DEVELOPMENT, TROPHOBLAST STEM CELLS, HISTONE CHAPERONE

ISSCR Travel Award Recipient

TOPIC: EARLY EMBRYO

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A SINGLE-CELL GENE EXPRESSION ATLAS OF MUCOCILIARY EPITHELIAL DEVELOPMENT

Sedzinski, Jakub - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Lee, Julie - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, Denmark*
Møller, Andreas Fønss - *Functional Genomics and Metabolism Unit, University of Southern Denmark, Odense, Denmark*
Chae, Shinhyeok - *Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea*
Bussek, Alexandra - *Novo Nordisk Foundation Center for Stem Cell Medicine, University of Copenhagen, , Denmark*
Kwon, Taejoon - *Department of Biomedical Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea*
Natarajan, Kedar Nath - *Functional Genomics and Metabolism Unit, University of Southern Denmark, Odense, Denmark*

Abstract: The vertebrate mucociliary epithelium acts as the critical barrier against infectious agents and pollutants. Various cell types of the mucociliary epithelium enable continuous airway clearing, and their defects lead to asthma, cystic fibrosis, and chronic obstructive pulmonary diseases. Here, using Xenopus embryonic tissue, we present a single-cell expression atlas of mucociliary epithelium during development, from pluripotent early blastula stages to fully established late tailbud stages. We identify novel transitory cell states, uncover their temporal ordering and fate-choice prior to terminal specification into known cell types. Using in silico lineage inference and in-situ HCR validation, we reconstruct developmental transitions and validate spatio-temporal cell type ordering over entire mucociliary epithelial development. This study provides a comprehensive characterization of developing mucociliary epithelium with new insights into cell fate transitions.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729)
Keywords: mucociliary epithelium, scRNAseq, Xenopus

TOPIC: GERMLINE

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TARGETED INSERTION OF CPG-FREE SSDNA INDUCES HERITABLE CG ISLAND METHYLATION RESPONSE (CIMR) IN MICE

Hussey, Deborah - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Shokrani, Alireza - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Lizhar, Elizabeth - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Tsark, Walter - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Wu, Xiwei - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Kang, Tae Hyuk - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Riggs, Arthur - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*
Tompkins, Joshua - *Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA, USA*

Abstract: Epigenetic processes, such as DNA methylation (DNAme), are fundamentally important for cellular differentiation. Throughout development, the epigenome is highly dynamic and is a driving factor for much of cellular differentiation, of which loss of proper regulation is associated with aging and disease. Epigenome engineering in pluripotent stem cells (PSCs) is challenging, and it is unclear which forms of editing are stably inherited through development, and which edits may alter cell behavior and developmental outcomes. Here, we report that a CpG island (CGI) site-specific integration of synthetic, CpG-free single-stranded DNA (ssDNA) induces widespread de novo methylation at surrounding CpGs, as shown in a past report using plasmid-based integration in human embryonic stem cells (hESCs). In a separate study, we also report on the restriction of this CGI Methylated Response (CIMR) to the primed state of pluripotency. While initially developed as a technique for heritable, larger scale DNAme edits in vitro, we report here on the technique employed in vivo, where integration of CpG-free ssDNA into a CGI by injection at the two-cell stage of murine development induces a CIMR. Unexpectedly, the DNAme edits were observed to be transgenerationally inherited over at least four generations without loss. The inheritance of epigenetic memory through the germline has been difficult to explain due to the global loss of DNAme during primordial germ cell (PGC) development, shortly after fertilization. This is in active area of research. Collectively, this method not only allows for the generation and study of epigenetic models of development and disease in PSCs, but also demonstrates progress made towards the study of transgenerational inheritance of engineered DNAme in mice.

Keywords: Epigenetic engineering, CpG Island, Primed pluripotency

TOPIC: IMMUNE SYSTEM

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HETEROGENITY OF HUMAN IPSC-DERIVED MSCS AND THEIR TISSUE-DERIVED COUNTERPARTS: A COMPARITIVE ANALYSIS OF THE MSC SECRETOME

Hodgson-Garms, Margeaux - *Materials Science and Engineering, Monash University, Clayton, Australia*
Frith, Jessica - *Materials Science and Engineering, Monash University, Melbourne, Australia*
Kelly, Kilian - *Cynata Therapeutics, Melbourne, Australia*
Martino, Mikaël - *Australian Regenerative Medicine Institute, Australian Regenerative Medicine Institute, Melbourne, Australia*

Abstract: Mesenchymal stromal cells (MSCs) are currently the subject of over 1300 clinical trials. However, they are primarily sourced from tissue donations, meaning that effective clinical translation is hampered by a lack of available donors, donor-donor variation, and the requirement to substantially expand isolated cells before reaching clinical doses. To address this, MSCs have been derived from induced pluripotent stem cells (iMSCs). While experimental and clinical data on iMSCs has produced promising results, questions still exist about the comparability of iMSCs to their tissue-derived counterparts. This is further complicated by the inherent heterogeneity of MSC populations and the lack of comprehensive data on MSC heterogeneity between or within populations. This study has compared 13 MSC populations, including multiple batches of clinical grade and commercially available iMSCs, alongside tissue-derived MSCs from bone marrow, adipose tissue and umbilical cord. As paracrine activity underpins much of MSC clinical utility LC-MS/MS was used to profile the MSC secretome under both resting and inflammatory licensed conditions. Analysis identified conserved secretome profiles of resting and licensed phenotypes as well as identifying factors uniquely secreted by individual subsets of MSCs. Resting MSCs secreted proteins mapped to angiogenic, wound healing and pro-cell survival processes whilst inflammatory-licensed MSCs upregulated secretion of proteins mapped to antigen presentation and T cell regulatory processes. Major factors driving the paracrine activity of inflammatory-licensed MSCs included IDO, HLA and CXCL molecules, and ICAM1. This dataset also permitted a comparison of the degree of donor and tissue-based heterogeneity, indicating significantly less heterogeneity between iMSC batches, and identified MSC subsets in which secretion of specific factors is enhanced. The scope of this proteomic profiling permits the identification of generalised factors of the MSC phenotype which may help in the specification of their defining criteria, but also characterises tissue and source-based heterogeneity which could be leveraged to enhance predictability of MSC efficacy. Furthermore, these results support our fundamental understanding of iMSCs as a promising alternative in the clinic.

Keywords: Multipotent mesenchymal stromal cells (MSC), Secretome, iPSC-derived / iMSC

TOPIC: NEURAL

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ENGINEERED SOX17 DRIVES THE DIRECT AND FAST REPROGRAMMING TOWARDS NEURAL STEM CELLS WITHOUT A DETOUR TO PLURIPOTENCY

Weng, Mingxi - *School of Biomedical Sciences, The University of Hong KongVejdani, Seyyedeh - School of Biomedical Sciences, The University of Hong Kong*
Hu, Haoqing - *School of Biomedical Sciences, The University of Hong Kong*
Graus, Matthew - *The Centenary Institute, The David Richmond Laboratory for Cardiovascular Development: Gene Regulation and Editing Program, Camperdown, Australia*
Tan, Daisylyn - School of Biomedical Sciences, The University of Hong KongHo, Derek - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Langer, Jakob - *School of Biomedical Sciences, The University of Hong Kong*
Holzner, Markus - *School of Biomedical Sciences, The University of Hong Kong*
Huang, Yuhua - *School of Biomedical Sciences, The University of Hong Kong*
Lai, Cora - School of Biomedical Sciences, The University of Hong KongLing, Guang Sheng - *School of Biomedical Sciences, The University of Hong Kong*
Francois, Mathias - *The Centenary Institute, The David Richmond Laboratory for Cardiovascular Development: Gene Regulation and Editing Program, Camperdown, Australia*
Jauch, Ralf - *School of Biomedical Sciences, The University of Hong Kong*

Abstract: Cell fate conversion by ectopic expression of transcription factors provides strategies for cell-based therapies. Induced neural stem cells (iNSCs) are promising cell sources for neurological disease modeling and regenerative medicine that bypass shortcomings of pluripotent cells such as tumorigenicity, tedious protocols and a neonatal phenotype. However, methods to transdifferentiate somatic cells into iNSCs are often slow with low reprogramming efficiency, and it is unclear whether cells go through a pluripotent state. Here, we describe an iNSC reprogramming approach using an engineered endodermal factor Sox17 (eSox17). eSox17 can efficiently generate self-renewing and tri-potent iNSCs from mouse embryonic, adult and aged fibroblasts while wild-type Sox2 and Sox17 fail. We defined reprogramming roadmaps using sensitive lineage reporters combined with genome-wide analysis and contrast the routes towards pluripotent versus neural stem cells. We further analyzed search behavior of eSox17 in chromatin on single-molecule level, defined molecular determinants for its reprogramming activity and compared DNA and protein interactions compared to the incapacitated factors Sox2 and Sox17. In the human system, we have directly reprogrammed human fibroblasts towards iNSCs with eSox17. To take one step closer to neuromuscular disease modeling, spinal cord motor neurons are generated from human iNSCs and co-cultured with muscle cells. Overall, our work reveals new molecular and physiological mechanisms for iNSC reprogramming with eSox17. We will discuss our efforts to apply this method in the human system to model neurodegenerative diseases.

Funding Source: Health and Medical Research Fund, the Research Grants Council of Hong Kong General Research Fund, the National Natural Science Foundation of China, the Innovation Technology Commission Funding
Keywords: cell fate conversion, induced neural stem cell, engineered Sox17

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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MAINTENANCE OF MESENCHYMAL STEM CELLS IN XENOGENIC-FREE MEDIUM

Mazzella, Matteo - *Biological Sciences, Oakland University, Shelby Township, MI, USA*
Walker, Keegan - *Biological Sciences, Oakland University, Rochester, MI, USA*
Cormier, Christina - *Biological Sciences, Oakland University, Rochester, MI, USA*
Kapanowski, Michael - *Biological Sciences, Oakland University, Rochester, MI, USA*
Govind, Yash - *Biological Sciences, Oakland University, Rochester, MI, USA*
Saifee, Azeem - *Biological Sciences, Oakland University, Rochester, MI, USA*
Chaudhry, Rasul - *Biological Sciences, Oakland University, Rochester, MI, USA*

Abstract: Mesenchymal stem cells (MSCs) show tremendous therapeutic and tissue engineering potential. However, their use remains limited due to poor growth and self-renewal as well as differentiation capabilities. They also progressively become senescent or undergo cell cycle arrest upon passaging. We investigated the commercial media with or without fetal bovine serum (FBS) supplementation to ascertain the effect of these media on the properties of MSCs. Primitive (p) MSCs were cultured to low (LP) (Passage 3) and high (HP) passages (Passage 20) using FBS and xeno-free (XF) media and analyzed for rate of proliferation, colony-forming efficiency (CFU), expression of MSC surface markers and trilineage differentiation potential. pMSCs showed fibroblastoid morphology in FBS medium but became larger and more elongated in HP than LP. Whereas they displayed small fibroblastoid morphology at both LP and HP in XF medium. Doubling time of pMSCs progressively increased upon passaging in FBS medium but remained low and constant in XF medium. Expression of CD90 and CD49f was significantly reduced to 82.6% and 1.4%, respectively in HP cells in FBS medium. CFU and cell proliferation were also reduced considerably in HP cells grown FBS medium. Both LP and HP cells cultured in XF and LP in FBS media showed a significantly higher differentiation towards chondrogenic and osteogenic lineages but HP cells cultured in FBS showed greater induction towards adipogenic lineages. RNA-seq analysis revealed an upregulation CCND2 and PSMD7 in LP and HP cells grown in XF medium but they were downregulated in HP cells cultured in FBS medium. However, six genes most upregulated in HP cells grown in FBS medium were mostly long-noncoding RNAs. Furthermore several genes (i.e. PCNA, POLA1 and RFC1) involved in DNA replication were upregulated in LP and HP cells cultured in XF medium. Moreover, many senescence genes such as IL1A, ITPR2, PTEN and CXCL8 were upregulated in HP cell grown in FBS medium. MNase-seq analysis showed that senescence genes tightly bound histones at the TSS limiting the expression in XF medium cells. Histones were not tightly bound in the same senescence genes in cells grown in FBS medium. These findings provide a new insight into the effect of culturing

medium composition and passaging on the stemness and other properties of MSCs.

Keywords: Mesenchymal stem cells, Proliferation, Senescence

TOPIC: PLURIPOTENT STEM CELLS

912

INHIBITION OF N-MYRISTOYLTRANSFERASE PROMOTES NAIVE PLURIPOTENCY IN MOUSE AND HUMAN PLURIPOTENT STEM CELLS

Horie, Kyoji - *Department of Physiology II, Nara Medical University, Kashihara, Japan*

Abstract: Naive and primed states are distinct states of pluripotency during early embryonic development that can be captured and converted to each other in vitro. To elucidate the regulatory mechanism of pluripotency, we performed recessive genetic screening of homozygous mutant mouse embryonic stem cells (mESCs) and found that N-myristoyltransferase (Nmt) suppression promotes naive pluripotency. The disruption of Nmt1 in mESCs conferred resistance to differentiation, and Nmt suppression in mouse epiblast stem cells (mEpiSCs) promoted the conversion from the primed to the naive state. This effect was independent of Src, which is a major substrate of Nmt and is known to promote mESC differentiation. Nmt suppression in naive-state human induced pluripotent stem cells (hiPSCs) increased the expression of the naive-state marker. These results indicate that Nmt is a novel target for regulating naive pluripotency conserved between mice and humans.

Keywords: N-myristoyltransferase, naive state, primed state

914

A DCAS9 BASED METHOD CAN IDENTIFY NOVEL REGULATORS OF NANOG AND PLURIPOTENCY

Burramsetty, Arun Kumar - *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*
Nishimura, Ken - *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*
Hisatake, Koji - *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*

Abstract: Even after decades of studying pluripotency, lack of defining conditions for maintaining pluripotency long-term and efficient production of iPS cells indicate our inadequacy in understanding pluripotency. Employing subtle methods of screening to identify pluripotency-related factors could be one reason. Identification of CRISPR-Cas9 let researchers approach genome loci with ease and comfort, and utilization of dCas9 (deactivated Cas9) enables locus-specific pull-down of DNA, RNA as well as proteins, called CAPTURE method. It helped to understand regulatory mechanisms comprehensively at any given locus. To identify proteins/mechanisms that associate with pluripotency; we utilized CAPTURE method to deduce transcriptional regulation at Nanog promoter in mouse embryonic stem cells (mESC). dCas9-mediated pull down of Nanog promoter revealed more than 300 proteins including some known promoter binding proteins like Trim28, Thrap3 and Bclaf1 suggesting the complexity of regulatory mechanism at the promoter regions. Based on functional ontology; six proteins (Thrap3; Bclaf1; Psip1; Park7; Msh6 and Fubp1) were chosen and tested for their role in pluripotency. While most of the chosen proteins showed binding to Nanog promoter in a ChIP-qPCR analysis. Knockdown (KD) of Fubp1 showed significant loss of Nanog and Oct4 (RT-qPCR) gene expression in pluripotency maintenance conditions. When the LIF was removed, KD of Fubp1 behaved as expected, but interestingly KD of Thrap3 and Park7 inhibited the loss of Nanog, and Oct4 significantly suggesting their role in pluripotency maintenance and differentiation induction. On the other hand, KD of all proteins except Fubp1 showed smaller EB's compared to control while only KD of Bclaf1 and Msh6 showed significant effect of EB development. Hence, unlike previous approaches, our single locus-based approach identified many new proteins regulating pluripotency. These results re-instigate that our CAPTURE of Nanog promoter successfully identified new regulatory proteins involved in Nanog regulation and pluripotency, suggesting the robustness of this approach in comprehensive understanding of transcription regulation at any given locus. When further studied, these proteins could give interesting insights towards pluripotency.

Keywords: CAPTURE, Nanog Promoter, Pluripotency

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: CARDIAC

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

204

VASCULAR ENDOTHELIAL CELLS AND ORGANOIDS DERIVED FROM CRISPR/CAS9-ENGINEERED PATIENT IPSCS PROVIDE LONG-TERM THERAPEUTIC CORRECTION OF HEMOPHILIA A

Kim, Gyeongmin - *Department of Biotechnology, Korea University, Seoul, Korea*
Son, Jeong Sang - *Department of Biotechnology, Korea University, Seoul, Korea*
Park, Chul-Yong - *Department of Physiology, Yonsei University College of Medicine, Seoul, Korea*
Lee, Gyunggyu - *Department of Biotechnology, Korea University, Seoul, Korea*
Park, Ji Young - *Department of Biotechnology, Korea University, Seoul, Korea*
Kim, Hyo Jin - *Biotechnology, Korea University, Seoul, Korea*
Chi, Kyun Yoo - *Department of Biotechnology, Korea University, Seoul, Korea*
Woo, Dong-Hun - *Department of Stem Cell Biology, NEXEL Co., Ltd., Seoul, Korea*
Han, Choongseong - *Department of Stem Cell Biology, NEXEL Co., Ltd., Seoul, Korea*
Kim, Sang Kyum - *College of Pharmacy, Chungnam National University, Daejeon, Korea*
Park, Han-Jin - *Department of Predictive Toxicology, Korea Institute of Toxicology, Daejeon, Korea*
Kim, Dong-Wook - *Department of Physiology, Yonsei University College of Medicine, Seoul, Korea*

Kim, Jong-Hoon - *Department of Biotechnology, Korea University, Seoul, Korea*

Abstract: Hemophilia A (HA) is an X-linked bleeding disorder caused by mutations in the F8 gene encoding the blood coagulation factor VIII (FVIII). The most common cause of severe HA is chromosomal inversions of introns 1 and 22 of the F8 gene, which impact more than 60% of HA patients. According to recent research, FVIII is predominantly generated in liver sinusoid endothelial cells. In this study, we used F8-defective human induced pluripotent stem cells (F8d-HA hiPSCs) from an HA patient and F8-corrected (F8C) HA hiPSCs, which had previously established by CRISPR/Cas9 genome engineering of F8d-HA hiPSCs. We produced a highly enriched population of CD157+ endothelial cells (ECs) from F8c-HA hiPSCs. These ECs had a significant level of FVIII activity, which was not observed in F8d-HA hiPSC-ECs. After transplantation, the genome-engineered F8c-HA hiPSC-ECs significantly prevented bleeding episodes in HA mice and restored plasma FVIII activity. Notably, EC grafts with a high dose reduced the bleeding time in HA mice throughout repeated consecutive bleeding challenges. In addition, the engrafted ECs survived in HA mice for more 3 months and corrected bleeding phenotypes in lethal wounding trials. These results indicate that F8c-HA hiPSC-ECs retained the ability to produce long-lasting therapeutic level of FVIII to correct the bleeding phenotype of HA. We also assembled three different cell types in microwell devices to create F8c-HA hiPSC-derived 3D liver organoids (LOGDs) and demonstrated their therapeutic impact in HA mice. Our data show that combining genome-engineering and iPSC technology represents a novel therapeutic modality for treating HA via autologous cell-mediated gene therapy.

Funding Source: This work was supported by the Technology Innovation Program (20009350) funded by MOTIE, Korea and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2018M3A9H1019504).
Keywords: Hemophilia A, Endothelial cells, Genome-editing

TOPIC: ETHICAL, LEGAL, AND SOCIAL ISSUES; EDUCATION AND OUTREACH

206

UNTESTED STEM CELL INTERVENTIONS IN THE US: THE NEED FOR STRINGENT REGULATION AND ENFORCEMENT

Foong, Chee (Patrick) - *Law, Western Sydney University, Sydney, Australia*

Abstract: In the U.S., clinics offering stem cell therapies not approved by the U.S. Food and Drug Administration (FDA) grew four-fold in five years, according to recent research performed by the University of California, Irvine, led by a bioethicist, Professor Leigh Turner. These putative medical treatments lack concrete evidence of safety and efficacy. In spite of extensive FDA regulations on cell-based interventions, marketplace expansion occurs. While there are stem cell products that fall within the same surgical exception and minimally manipulated cellular products used in a homologous manner that do not require premarketing review and licensure by the FDA, a lot of these interventions are classified as biologics, drugs or medical devices that need safety and efficacy testing in clinical trials as well as FDA premarketing authorisation. Thus, there is a strong need for stringent regulation and significant enforcement steps by regulatory bodies like the FDA, the Federal Trade Commission (FTC) and others. This presentation will examine the laws and enforcement measures to prevent unproven interventions and advance appropriate care consistent with best clinical practice.

Funding Source: None.
Keywords: Law, Ethics, Regulation

TOPIC: HEMATOPOIETIC SYSTEM

208

TIME- AND LINEAGE-RESOLVED SCRNA-SEQUENCING REVEALS THE TRANSCRIPTIONAL DRIVERS OF T LINEAGE SPECIFICATION FROM PLURIPOTENT STEM CELLS

Major, Matthew C. - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Michaels, Yale - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Bonham-Carter, Becca - *Math Department, University of British Columbia, Vancouver, BC, Canada*
Greenstreet, Laura - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Forrow, Aden - *Math Department, University of British Columbia, Vancouver, BC, Canada*
Castle, Elizabeth - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Edgar, John - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Zimmerman, Carla - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*
Rios, Myriam Iliana Ibanez - *Institute for Research in Immunology and Cancer, Universite de Montreal, Montreal, QC, Canada*
Knapp, David - *Institute for Research in Immunology and Cancer, Universite de Montreal, Montreal, QC, Canada*
Schiebinger, Geoffrey - *Math Department, University of British Columbia, Vancouver, BC, Canada*
Zandstra, Peter - *School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada*

Abstract: While T cells have high efficacy as therapeutics for blood malignancies and are promising for the treatment of other diseases, high manufacturing costs and batch variability pose a challenge for widespread adoption of T cell therapy. The recent development of feeder-free in vitro platforms capable of differentiating pluripotent stem cells (PSCs) to T cells may alleviate these challenges. The self-renewing capabilities of PSCs and scalability of these in vitro systems would reduce the batch variability and high costs associated with T cell therapy. Furthermore, these systems give us an unprecedented opportunity to investigate T cell development under defined conditions and thus may reveal fundamental biological mechanisms that would otherwise be obfuscated. Here, using our lab's PSC to T cell differentiation platform, we performed time course single-cell RNA sequencing and lineage barcoding to investigate the fate bifurcation and transcriptional changes underlying differentiation of progenitor T cells from PSC-derived hemogenic endothelial cells. By applying the trajectory inference method Waddington Optimal Transport (WOT) to the dataset, we determined when fate decisions occur in real time and the specific progenitor populations giving rise to terminal cell types. We identified fate biases along with overlapping progenitor populations for blood progenitor cells through direct analysis of the lineage barcoding data and by enhancing the WOT analysis with clonal information using LineageOT. Com-

bining the LineageOT analysis with SCENIC regulons revealed a number of transcription factors associated with T cell emergence from multipotent cells including MYC. This analysis uncovered blood differentiation biases as well as underlying transcriptional changes that are exploitable targets for improving the in vitro production of T cells from PSCs while providing insights into development of the blood system.

Funding Source: Canadian Institute for Health Research Michael Smith Foundation Natural Sciences and Engineering Research Council of Canada Notch Therapeutics Stem Cell Network Wellcome Leap Human Organs, Physiology, and Engineering Zymeworks
Keywords: Blood and lymphocyte development, Lineage tracing sequencing, Single-cell RNA sequencing

TOPIC: MUSCULOSKELETAL

210

CRISPR/CAS9 EDITING OF DIRECTLY REPROGRAMMED MYOGENIC PROGENITORS RESTORES DYSTROPHIN EXPRESSION IN A MOUSE MODEL OF MUSCULAR DYSTROPHY

Domenig, Seraina A. - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
Bundschuh, Nicola - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
Lenardič, Ajda - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
Ghosh, Adhideb - *Functional Genomics Center Zurich, Zürich, Switzerland*
Kim, Inseon - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
Qabrati, Xhem - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
D'Hulst, Gommaar - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*
Bar-Nur, Ori - *Department of Health Sciences and Technology, ETH Zürich, Schwerzenbach, Switzerland*

Abstract: Genetic mutations in dystrophin manifest in Duchenne muscular dystrophy (DMD), the most prevalent form of a genetically inherited muscle disease. Dystrophin is expressed in myogenic stem cells and fibers, playing a critical role in maintaining skeletal muscle structure, regeneration and function. Here we report on direct reprogramming of fibroblasts derived from the Dmdmdx or Dmdmdx-4Cv mouse models of Duchenne muscular dystrophy into induced myogenic progenitor cells (iMPCs) by transient MyoD overexpression in concert with small molecule treatment. DMD iMPCs proliferated extensively in vitro while expressing canonical skeletal muscle stem and progenitor cell markers including Pax7, Sox8 and Myf5. Additionally, DMD iMPCs readily gave rise to highly contractile and multinucleated myofibers that expressed a suite of mature skeletal muscle markers however lacked dystrophin expression. Utilizing an exon-skipping based approach with CRISPR/Cas9, we report on genetic correction of the dystrophin mutation in Dmdmdx or Dmdmdx-4Cv iMPCs and subsequent restoration of dystrophin protein expression in vitro. Furthermore, engraftment of genetically corrected DMD iMPCs into limb muscles of dystrophic mice restored dystrophin expression in vivo and contributed donor-derived cells to the muscle stem cell reservoir. Collectively, our findings report on a novel in vitro stem cell-based model for DMD and further establish an approach to restore dystrophin expression in vivo via a combination of direct reprogramming, gene editing and cell transplantation.

Keywords: Duchenne muscular dystrophy, Direct lineage reprogramming, CRISPR/Cas9 editing

TOPIC: NEURAL

212

ROLE OF PENTRAXIN-3 AND MICRO-RNA-21-5P IN MODULATING ACTIVATED HUMAN MICROGLIA BY EXTRACELLULAR VESICLES FROM HUMAN IPSC-DERIVED NEURAL STEM CELLS

Madhu, Leelavathi N. - *Molecular and Cellular Medicine, Institute for Regenerative Medicine, Texas A&M Health Science Center, College Station, TX, USA*
Upadhya, Raghavendra - *Molecular and Cellular Medicine, Institute for Regenerative Medicine, Texas A&M Health Science Center, College Station, TX, USA*
Shetty, Ashok - *Molecular and Cellular Medicine, Institute for Regenerative Medicine, Texas A&M Health Science Center, College Station, TX, USA*

Abstract: Extracellular vesicles (EVs) released by human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSC-EVs) have potent antiinflammatory and neurogenic properties and are proficient in permeating the entire brain after intranasal administration. Moreover, proteomics and small RNA sequencing studies have revealed multiple therapeutic proteins and miRNAs in their cargo, making them an attractive off-the-shelf product for treating neurodegenerative diseases. However, their ability to directly modulate activated human microglia or specific proteins and miRNAs mediating their antiinflammatory effects are unknown. Therefore, we investigated the proficiency of hiPSC-NSC-EVs to modulate activated human microglia in vitro and then probed the role of a highly enriched protein (pentraxin 3) and a highly enriched miRNA (miR-21-5p) in mediating their antiinflammatory effects. We first generated mature microglia from hiPSCs (iMicroglia), which expressed multiple microglia-specific markers. iMicroglia responded to lipopolysaccharide (LPS) or interferon-gamma challenge by upregulating tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) mRNAs and releasing increased concentrations of TNF-α and IL-1β proteins into the culture media. iMicroglia also exhibited proficiency to phagocytose amyloid beta (Aβ). Notably, the addition of hiPSC-NSC-EVs to LPS-stimulated iMicroglia (proinflammatory microglia) cultures dose-dependently decreased TNF-α and IL-1β mRNA expression in iMicroglia and the release of TNF-α and IL-1β by them. However, the antiinflammatory activity of hiPSC-NSC-EVs on LPS-stimulated microglia significantly diminished when PTX3 or miR-21-5p concentration was reduced in EVs through siRNA approaches. The results suggest that hiPSC-NSC-EVs can modulate the proinflammatory human microglia into noninflammatory phenotypes, and PTX3 and miR-21-5p are among the constituents of hiPSC-NSC-EVs that mediate their antiinflammatory activity. The results also imply the utility of EVs shed by hiPSC-NSCs to reduce neuroinflammation in neurodegenerative diseases and highlight the potential utility of PTX3 and miR-21-5p overexpression in hNSC-EVs to enhance their antiinflammatory activity further.

Funding Source: Supported by a grant from the National Institute of Neurological Disorders and Stroke (1R01NS106907 to A.K.S)

Keywords: Antiinflammatory effects, Extracellular vesicles, Human neural stem cells

214

INJECTABLE 3D CELLULAR MICROCULTURES ENABLE INTRACEREBRAL GRAFTING OF DOPAMINE NEURONS DIRECTLY REPROGRAMMED FROM ADULT HUMAN FIBROBLASTS

Kajtez, Janko - *Experimental Medicine, Lund University, Lund, Sweden*
Nilsson, Fredrik - *Experimental Medicine, Lund University, Lund, Sweden*
Cepeda-Prado, Efrain - *Experimental Medicine, Lund University, Lund, Sweden*
Bruzelius, Andreas - *Experimental Medicine, Lund University, Lund, Sweden*
Birtele, Marcella - *Experimental Medicine, Lund University, Lund, Sweden*
Barker, Roger - *Clinical Neurosciences, University of Cambridge, UK*
Fiorenzano, Alessandro - *Experimental Medicine, Lund University, Lund, Sweden*
Rylander Ottosson, Daniella - *Experimental Medicine, Lund University, Lund, Sweden*
Parmar, Malin - *Experimental Medicine, Lund University, Lund, Sweden*

Abstract: Recent breakthroughs in cellular reprogramming have paved the way for direct conversion of human fibroblasts into induced dopaminergic neurons (iDANs) that can be used for disease modeling and potentially also provide a quicker source of cells for transplantation-based therapies. With these advantages, direct reprogramming revives the hope of personalized cell replacement therapy on demand for neurological disorders such as Parkinson's disease (PD). However, when cultured in conventional monolayers, iDANs generated from fibroblasts do not survive the transplantation well. Therefore, there is an urgent need for solutions that will enable successful intracerebral grafting of sensitive postmitotic cells such as iDANs. To address this issue, we present a novel 3D in vitro reprogramming strategy of patient-specific fibroblasts into iDAN spheroids (iDANoids). To achieve this, fibroblasts are seeded on arrays of ultra-low attachment microwells. Within each microwell, the cells self-assemble into a 3D structure and are converted into dopaminergic neurons using a set of 6 transcription factors together with suppression of the REST complex. As a result, we can simultaneously create thousands of iDANoids with uniform but tunable size. We show that the converted cells are functionally active and express neuronal (and dopaminergic) markers both at mRNA and protein levels. Importantly, iDANoids can be easily harvested without the need for enzymatic or mechanical dissociation and injected into the brain of a 6-OHDA PD rat model for minimally invasive intracerebral grafting. This results in functional neuron and dopamine rich grafts after 8 weeks. We are currently performing further assessments on long term survival, function, and integration in vivo. In summary, we present here a versatile but simple 3D reprogramming platform that provides increased cell-cell interactions in a more physiologically relevant environment and that results in formation of iDANs that survive and functionally integrate into the host circuitry.

Keywords: direct reprogramming, 3D culture, transplantation

TOPIC: PLURIPOTENT STEM CELLS

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IDENTIFICATION OF CANCER-RELATED MUTATIONS IN HUMAN PLURIPOTENT STEM CELLS AND THEIR NEURAL PROGENY USING RNA-SEQUENCING ANALYSIS

Lezmi, Elyad - *Genetics, The Hebrew University, Jerusalem, Israel*
Avior, Yishai - *Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel*
Jung, Jonathan - *Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel*
Eggan, Kevin - *Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*
Benvenisty, Nissim - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Human pluripotent stem cells (hPSCs) are known to acquire chromosomal aberrations in culture, affecting their growth as well as their tumorigenic potential. We have recently shown using whole exome sequencing and RNA-sequencing, that hPSCs acquire cancer-related point-mutation in the tumor suppressor gene TP53, suggesting the presence of cancer-related mutations also in other genes. Using our established methodology for the identification of cancer-related point-mutations using RNA-sequencing, we analyzed published data of the prevalent hPSC lines; WA01 and WA09, to investigate whether hPSCs acquire additional cancer related mutations in culture. By comparing 172 RNA-sequencing samples, originating from 44 studies of 40 different research groups, to early passage WES of the same cell lines, we identified cancer-related mutations in 33% of the samples. While TP53 remains the most mutated gene in hPSCs, with four individual recurrent mutations, we have identified less frequent events also in the genes EGFR, PATZ1, and CDK12. Our most recent analysis identified cancer-related point-mutations not only in undifferentiated hPSCs, but also in their neural progenies. Importantly, we highlight the persistent expression of dominant-negative mutant alleles in differentiated hPSCs-derived cells. Thus, we show the acquisition of pathogenic mutations characteristic to human tumors, during the culturing of hPSCs, and present a bioinformatic pipeline for the identification of such mutations using RNA-sequencing.

Keywords: Pluripotent stem cells, point-mutations, cancer-related genes

TOPIC: EYE AND RETINA

916

FUNCTIONAL CELL DISPLACEMENT OF DISEASED CORNEAL ENDOTHELIAL CELLS WITH INDUCED PLURIPOTENT STEM CELL-DERIVED CORNEAL ENDOTHELIAL-LIKE CELLS IN A CORNEAL ENDOTHELIAL DYSFUNCTION ANIMAL MODEL

Lee, Hun - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Park, Yoonkyung - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*

So, Seongjun - *Biomedical Science, CHA University, Gyeonggi, Korea*
Sunwoo, Jeong Hye - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Lee, Whanseo - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Kim, Jin - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Kim, Jae Yong - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Tchah, Hungwon - *Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
Kang, Eunju - *Biomedical Science, CHA University, Gyeonggi, Korea*

Abstract: We evaluate the efficacy and safety of transplantation of corneal endothelial cells (CECs) differentiated from fibroblasts-derived induced pluripotent stem cells (iPSCs) into a corneal endothelial dysfunction animal model. iPSCs were generated from fibroblasts using the iPS Reprogramming Kit and cultured under standard iPSC culture conditions. The characteristics of the iPSCs were analyzed using immunocytochemistry, teratoma assay, and quantitative real-time PCR (qRT-PCR). Differentiation of iPSCs into CECs via neural crest cells (NCC) was induced using a conditioned medium in vitro. After culturing NCC for 10 days with human endothelium serum-free media, corneal endothelial cell markers were detected by immunofluorescence. The gene expression of iPSC-derived CECs was measured by qRT-PCR. The cultured iPSC-derived CECs were transplanted into the anterior chamber of rabbits by direct cell injection. The iPSCs expressed the PSC markers, OCT4, SOX2, TRA-1-60, and NANOG. In addition, the iPSCs formed teratomas and differentiated into a 3-germ layer lineage. After 10 culturing iPSC-derived NCC for 10 days with human endothelium serum-free media, corneal endothelial cell-related markers, including zonula occludens-1 (ZO-1) and Na+/K+ ATPase, were expressed in the iPSC-derived CECs and exhibited a well-preserved hexagonality. The expression of ATP1A1, COL8A1, and AQP1 mRNA was higher in iPSC-derived CECs, compared with NCC and iPSCs. In animal experiments, increased corneal transparency was achieved after anterior chamber injection of iPSC-derived CECs. PCR of genomic DNA extracts of the central cornea, which was enucleated 3 weeks after cell injection, revealed a human mitochondrial DNA band, whereas a negative band was noted in the untreated rabbit. This preclinical study validates a therapeutic approach using iPSC-derived CECs in vivo. Our findings demonstrate that iPSC-derived CECs represent a promising source for cell therapy in corneal endothelial dysfunction.

Funding Source: Korea Medical Device Development Fund grant (9991006821, KMDF_PR_20200901_0148), Korean Fund for Regenerative Medicine (21C0723L1-11), NRF-2020M3A9E4036527, Asan Institute for Life Sciences (2022IP0019-1, 2021IP0061-2)
Keywords: Induced pluripotent stem cell, Cornea, Corneal endothelial cells

TOPIC: KIDNEY

918

ESTABLISHMENT OF AN AUTOLOGOUS, HUMAN MULTI-ORGAN-CHIP PLATFORM FOR EVALUATION OF CELL THERAPIES

Durieux, Isabell - *Multi-Organ-Chips, TissUse GmbH / Charité Berlin, Berlin, Germany*
Marx, Uwe - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Horland, Reyk - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Volk, Hans-Dieter - *Immunology, BIH Center for Regenerative Medicine, Berlin, Germany*
Reine, Petra - *Nephrology and Transplantation, BIH Center for Regenerative Medicine, Berlin, Germany*
Dehne, Eva-Maria - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Accastelli, Enrico - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Erfurth, Hendrik - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Sonnenberger, Jacqueline - *Multi-Organ-Chips, TissUse GmbH, Berlin, Germany*
Jäger, Jonas - *Multi-Organ-Chips, Amsterdam UMC, Amsterdam, Netherlands*

Abstract: The clinical development of advanced human cell therapies suffers from a lack of adequate preclinical testing in laboratory animals. The informative value of such (humanized) animal trials are limited due to their phylogenetic distance to humans and, especially, their lack of a human immune system. Due to the histocompatibility mismatch between laboratory animals and the patient, challenges increase significantly once personalized regulatory T cell (Treg) therapy approaches for the prevention of transplant rejection are under evaluation. Adoptive transfer of Tregs is a promising therapeutic option to reshape intra-tissue immune imbalance in transplant patients. It aims at supporting long-term function of allografts by overcoming the challenge of undesired immune reaction by the recipient. Here, we used the HUMIMIC® multi-organ-chip platform to establish a next-generation human in vitro assay for predictive preclinical testing of Treg products. The platform enables co-culture of various human organ models but lacks blood micro-capillary vessel structures covered with human endothelial cells. For this purpose, we implemented a network of miniature vascularized channels in the organ compartments of the HUMIMIC® platform for two-organ co-culture exploring 3D printing tools and endothelial self-assembly processes. The organ models and endothelial cells were generated from iPSCs of two different individual HLA-tested healthy persons emulating the recipient and the donor background. Finally, we aimed to qualify a HUMIMIC® based next-generation transplant rejection assay to evaluate both, safety and efficacy of Treg products in a universal repeated dose long-term assay environment. Multi-organ-chip design and prototyping results are presented along with the results of iPSC-based differentiation of human endothelial cells, and kidney models for the establishment of the interconnected two-organ model. Furthermore, we present data on on-chip micro-vessel formation and co-culture over prolonged culture periods. Results will be discussed in the light of the assay potential to replace respective animal transplant models in use.

Funding Source: This work is supported by EU-H2020 “ReSHAPE” Project, Grant Agreement n. 825392.

Keywords: Human cell therapies, Multi-Organ-Chip platform, Kidney transplantation model

TOPIC: NEURAL

920

GENERATION OF AN ARTIFICIAL STEM CELL NICHE USING A MICROFLUIDICS-BASED 3D CELL CULTURE PLATFORM

Norton, Benjamin M. - *Otolaryngology - Head & Neck Surgery, Northwestern University, Chicago, IL, USA*
Matsuoka, Akihiro - *Otolaryngology - Head & Neck Surgery, Northwestern University, Chicago, IL, USA*

Abstract: Stem cell-replacement therapy represents a promising treatment strategy for conditions affecting the inner ear, including sensorineural hearing loss. We have previously developed differentiation and transplantation protocols for three-dimensional (3D) human pluripotent stem cell (hPSC)-derived otic neuronal progenitor (ONP) spheroids. Despite encouraging progress, poor in vivo survival, differentiation, and synaptogenesis of engrafted hPSC-derived ONP spheroids, due to the rapid depletion of neurotrophins, are barriers to clinical realization. To prevent the depletion of brain-derived neurotrophic factor (BDNF), which is fundamental to the generation and maturation of spiral ganglion neurons (SGNs), we investigated an approach combining sustained neurotrophin release and protein retention strategies on a microfluidics-based 3D cell culture platform. A functionalized nanofibrillar cellulose (NFC) hydrogel system was created by embedding stable-releasing POlyhedrin Delivery System (PODS®; Cell Guidance Systems, Cambridge, UK)-BDNF co-crystals in avidin-conjugated NFC hydrogel (GrowDex-A; UPM-Kymmene, Helsinki, FI) complexed with biotinylated anti-BDNF antibody. Microfluidic channels were filled with functionalized NFC hydrogel to generate an artificial stem cell niche in vitro. Induced pluripotent stem cell (iPSC)-derived ONP spheroids generated according to our pre-established protocol were transplanted into the artificial stem cell niche and were differentiated toward the SGN phenotype. Results indicate that iPSC-derived otic neuronal spheroids displayed improved survival and increased expression of mature SGN markers compared with otic neuronal spheroids cultured in control conditions. These results substantiate the importance of the stem cell niche and indicate that utilization of a functionalized NFC hydrogel system may improve in vivo survival, differentiation, and synaptogenesis of transplanted hPSC-derived ONP spheroids. Future studies will assess the electrophysiological properties of otic neuronal spheroids cultured in the artificial stem cell niche and investigate the feasibility of transplanting hPSC-derived ONP spheroids embedded in a functionalized NFC hydrogel system into murine cochleae.

Keywords: stem cell niche, microfluidics, inner ear regeneration

TOPIC: PLURIPOTENT STEM CELLS

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THE ROLES OF DERMATAN SULPHATE IN STEM CELLS

Ogura, Chika - *Department of Bioinformatics, Graduate School of Engineering, Soka University, Hachioji, Japan*
Hirano, Kazumi - *Molecular Neurophysiology Research Group, Biomedical Research Institute, The National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan*
Mizumoto, Shuji - *Department of pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan*
Yamada, Shuhei - *Department of pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan*
Nishihara, Shoko - *Department of Bioinformatics, Graduate School of Engineering, Soka University, Hachioji, Japan*

Abstract: Glycosylation is a one of the post-translational modifications. Glycans have many various structures and bind to proteins and lipids. Many proteins on the cell surface and secreted proteins are glycosylated and involve in important biological events. The signal regulation is one of them, in which signal ligands and receptors bind to specific glycan structures. Glycosaminoglycans (GAGs) are present as proteoglycans, consisting of GAGs and core proteins, and include heparan sulfate (HS), keratan sulfate (KS), and chondroitin sulfate (CS) / dermatan sulfate (DS). GAGs are sulfated and have a characteristic disaccharide repeating structure. Mouse embryonic stem cells (mESCs) maintain self-renewal and pluripotency. Various signals and growth factors are committed to maintenance of undifferentiated state and regulation of differentiation in mESCs. Previously, we and other groups reported that heparan sulphate contributes to maintenance of undifferentiated state and regulation of mESCs differentiation by promoting signals. It has been shown that CS is needed for pluripotency and differentiation of mESCs, while KS is a known marker of human ESCs / iPSCs. However, the function of DS in mESCs has not been revealed yet. Here, we performed (1) induction of neuronal differentiation in mESCs and human neural stem cells (hNSCs) with purified DS and (2) knockdown (KD) or overexpression (O/E) of the dermatan-4-O-sulfotransferase-1 (D4ST1), which transfers sulfate to the C-4 hydroxyl group of N-acetylgalactosamine of DS in mESCs. The addition of DS promoted the neuronal differentiation from mESCs and hNSCs. Furthermore, the neurite outgrowth was accelerated in mESCs but not in hNSCs by adding DS; instead, neuronal migration was promoted in hNSCs. These results revealed that DS promotes neuronal differentiation in both mouse and human stem cells. D4ST1 KD caused a decreased in undifferentiation state and self-renewal, and induction of endodermal differentiation by activation of Wnt signal in mESCs. In contrast, D4ST1 O/E rescued self-renewal and reduced activation of Wnt signal in mESCs. It is demonstrated that D4ST1 is required for undifferentiated state and self-renewal of mESCs. Our findings provide new insights into function of DS in stem cells.

Funding Source: This work was supported by the Japan Society for Promotion of Science (JSPS) KAKENHI [grant number JP18K06139 to S.N.] and Japan Science Technology Agency (JST)-Mirai Program [grant number JPMJMI18GB to S.N.]
Keywords: dermatan sulphate, self-renewal, neuronal differentiation

TOPIC: CARDIAC

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CELLULAR THERAPY FOR CHRONIC HEART FAILURE

Ong, Lay Ping - *Medicine - Cardiology, University of Cambridge, UK*
Colzani, Maria - *Medicine, University of Cambridge, UK*
Gambardella, Laure - *Medicine, Wellcome Trust Sanger Institute, Cambridge, UK*
Bertero, Alessandro - *Medicine, University of Torino, Italy*
Martinson, Amy - *Pathology, University of Washington, Seattle, WA, USA*
Murry, Charles - *Pathology, University of Washington, Seattle, WA, USA*
Sinha, Sanjay - *Medicine, University of Cambridge, UK*

Abstract: A heart attack results in irreversible cardiomyocyte loss, frequently leading to chronic heart failure with 50% 5-year mortality. We observed successful 'primary remuscularization' of the infarcted heart when human embryonic stem cell (hESC)-derived cardiomyocytes were introduced soon after myocardial infarction (MI). Outcomes were further improved by co-delivering hESC-derived epicardium. However, 'primary remuscularization' with hESC–derived cardiomyocytes alone showed no benefit in chronic heart failure. We investigated whether alternative cellular approaches e.g. species-matched (neonatal rat cardiomyocytes) or combination cellular therapy (hPSC-cardiomyocytes and hPSC-epicardium) could regenerate the chronically infarcted rat heart. We conducted a pilot study with chronically infarcted rodents. There were 3 study arms:- i) Vehicle-control, ii) P1/P2 neonatal rat cardiomyocytes (NRVM) and iii) hPSC-epicardium & cardiomyocytes (Epi+CM). All rats underwent temporary left anterior descending artery ligation to invoke MI. Four weeks after MI, only rats with fractional shortening (FS%) < 40% were determined to be in chronic heart failure and received intramyocardial cell injections. Subsequently, these rats underwent monthly echocardiographic follow-up for a total of 3 months post-injection. Overall, rats without any cell injections showed a persistent decline in their cardiac function. At 3 months post-cellular injection, NRVM demonstrated cardiac functional recovery whilst Epi+CM attenuated further cardiac dysfunction. Both NRVM and Epi+CM demonstrated robust cardiac grafts; Epi+CM's cardiac graft size=1.2±1.3% of LV whilst NRVM=2.7±1.9% of LV. These grafts displayed increased sarcomeric alignment, maturation, connexin-43 organization, and neovascularization when compared to previous reports. In conclusion, our pilot study showed that both species-matched therapy (NRVM) and combination cellular therapy (Epi+CM) ameliorated cardiac dysfunction in rats with chronic heart failure, albeit to different extents. Thus, cellular therapy holds clinical promise for 'remuscularising' chronically infarcted hearts. To validate these preliminary findings, a randomised preclinical study is highly warranted.

Funding Source: Wellcome Trust, British Heart Foundation, Addenbrooke's Charitable Trust
Keywords: Heart failure, Cardiac regeneration, Cellular Therapy

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: CARDIAC

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THE HUMAN FLT1 REGULATORY ELEMENT DIRECTS VASCULAR EXPRESSION AND MODULATES ANGIOGENESIS PATHWAYS IN VITRO AND IN VIVO

Stolper, Julian - *Cell Biology, Murdoch Children's Research Institute, Parkville, Australia*
Porrello, Enzo - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Elliott, David - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*
Ramialison, Mirana - *Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia*

Abstract: There is growing evidence that mutations in non-coding cis-regulatory elements (CREs) disrupt proper development. However, little is known about human CREs that are crucial for cardiovascular development. To address this, we bioinformatically filtered for cardiovascular CREs of interest, based primarily on the occupancy of the CRE by the homeodomain protein NKX2-5 and cardiac chromatin histone modifications. This search identified a highly conserved CRE within the FLT1 locus termed enFLT1. We show that the human enFLT1 is an enhancer capable of driving reporter transgene expression in vivo throughout the developing cardiovascular system of medaka. Deletion of the human enFLT1 enhancer (ΔenFLT1) triggered molecular perturbations in extracellular matrix organisation and blood vessel morphogenesis in vitro in endothelial cells derived from human embryonic stem cells and in vivo in medaka. In addition, the enhancer deletion triggered changes in FLT1 alternative isoform usage in medaka. These findings highlight the crucial role of the human FLT1 enhancer and its function as a regulator and buffer of transcriptional regulation in cardiovascular development.

Funding Source: This work was supported by NHMRC (1180905), the Royal Children's Hospital Foundation as well as the Stafford Fox Foundation.
Keywords: Regulatory elements, Enhanceropathies, Gene Regulation

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INVESTIGATING THE REDUCED CARDIOTOXICITY OF SINGLE PROTEIN ENCAPSULATION-BASED DOXORUBICIN USING IPSC-CARDIOMYOCYTES

Arzt, Madelyn - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Cui, Xiaojiang - *Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Gao, Bowen - *Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center,*

Los Angeles, CA, USA
Giuliano, Armando - *Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Huang, Faqing - *Department of Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, MS, USA*
Liu, Qizhi - *Department of Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Sharma, Arun - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Yu, Changjun - *Division of Chemistry & Chemical Engineering, California Institute of Technology, Pasadena, CA, USA*

Abstract: Cancer remains a leading cause of morbidity worldwide. The treatment of cancer continues to be effective with the broad use of small molecule chemotherapeutics such as doxorubicin (DOX), an anthracycline potent enough to combat multiple types of malignancies. However, DOX invades many bodily tissues including the heart during treatment, often leaving patients to face lifelong cardiovascular complications. Such severe side-effects present a need to develop heart-safe chemotherapeutic agents that still retain their anticancer efficacy. Here, we used hiPSC-derived cardiomyocytes (hiPSC-CMs), cancer cell lines, and a mouse model to evaluate the cardiotoxicity and anticancer efficacy of a novel single protein encapsulation (SPE)-based doxorubicin variant (SPEDOX), in which multiple DOX molecules are integrated into a single human serum albumin molecule. We assessed the utility of SPEDOX in mimicking DOX anticancer treatment by exposing human triple-negative breast cancer cell line BT549 to both wild-type (WT) DOX and SPEDOX. Visible cell death in the BT549 cells and immunofluorescence staining for albumin and DOX revealed the successful uptake of SPEDOX and its efficacy in killing cancer cells, similar to WT DOX. We next used hiPSC-CMs to serve as an in vitro model to characterize the cardiotoxic side effects of SPEDOX in comparison to those of WT DOX. Following a drug treatment time course, hiPSC-CMs exhibited significantly lower cytotoxicity values from SPEDOX than WT DOX, corresponding to a lower DOX fluorescence signal seen for hiPSC-CMs treated with SPEDOX. Functional assessment of hiPSC-CMs treated with WT DOX or SPEDOX showed significantly improved contractility during SPEDOX treatment, further establishing the reduced cardiotoxicity of SPEDOX in comparison to WT DOX. Finally, a DOX mouse model demonstrated lower SPEDOX accumulation in heart tissue when compared with WT DOX treatment. This study demonstrates the capacity of SPEDOX to alleviate the cardiotoxic side effects associated with DOX, while maintaining its anticancer potency. We believe that this SPE technology in combination with the use of hiPSCs can serve as a high-throughput platform for evaluating the cardiotoxicity of chemotherapeutic agents in a safe, pre-clinical setting.

Funding Source: American Heart Association Career Development Award 856987
Keywords: Cancer, Cardiotoxicity, Induced pluripotent stem cells

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HYDROGEL ASSISTED DOUBLE MOLDING OF 3D PRINT ENABLE PRESTRESS REGULATION OF STEM CELL DERIVED MICRO-HEART MUSCLE

Simmons, Daniel - *Biomedical Engineering, Washington University, Saint Louis, MO, USA*
Schuftan, David - *Biomedical Engineering, Washington University, St. Louis, MO, USA*
Guo, Jingxuan - *Mechanical Engineering & Materials Science, Washington University, St. Louis, MO, USA*
Oguntuyo, Kasoorelope - *Biomedical Engineering, Washington University, St. Louis, MO, USA*
Ramahdita, Ghiska - *Mechanical Engineering & Materials Science, Washington University, St. Louis, MO, USA*
Munsell, Mary - *Biomedical Engineering, Washington University, St. Louis, MO, USA*
Miller, Evan - *Chemistry, University of California, Berkeley, CA, USA*
Huebsch, Nathaniel - *Biomedical Engineering, Washington University, St. Louis, MO, USA*

Abstract: Resting tension plays an important role in heart function, and many engineered heart tissues are designed to induce cellular alignment and prestress. However, it is unclear how prestress induces cardiomyocyte maturation. To address this, we created 3D-printing based methods to control the geometry of pluripotent stem cell (iPSC)-derived micro-heart muscles (μHM), to determine how geometric induced prestress regulates electrophysiology. A double molding technique was used to replicate high-resolution 3D printed features into poly(dimethyl siloxane) (PDMS), overcoming the inability to replicate PDMS directly off 3D prints. Both agar and alginate-based double network gels were used to form negatives of 3D-prints. PDMS was then crosslinked within the gel negative molds. Gel crosslink concentration was critical for predicting feature transfer success. The resulting PDMS molds were used to create μHM. iPSC were differentiated into cardiomyocytes using small molecule manipulation of Wnt signaling, and seeded into rigid PDMS stencils to form μHM of multiple sizes. Increased μHM shaft length was hypothesized to increase tissue prestress based on a Comsol model of μHM uniform compaction. This was confirmed via increases in nuclear aspect ratio, along with increases in cell length without concurrent changes in width. Electrophysiology was then assessed optically using BeRST-1 voltage sensitive dye and GCaMP6f genetically encoded calcium indicator. Tissue prestress had global effects on EP. Studies in day 15 μHM with sodium current (INa) specific probe saxitoxin suggest that INa was absent in 3D tissues without geometrically-induced prestress. In contrast, studies with the hERG-specific probe, E4031, suggest potassium currents were less sensitive. qPCR showed no effects of tissue prestress on the sodium channel subunit SCN5A, suggesting changes in INa are regulated post-translationally. These results show hydrogels can replicate 3D prints into PDMS with high fidelity. Using this technique to modulate tissue geometry/prestress, we see global effects on EP. Pharmacology shows a prestress threshold is necessary for proper Nav1.5 function, while other channels are less sensitive.

Keywords: engineered heart tissue, cardiac electrophysiology, hydrogel

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HIPSC-CARDIOMYOCYTES FROM PATIENTS WITH INFANTILE MITOCHONDRIAL CARDIOMYOPATHY SHOW REDUCED MITOCHONDRIAL CAPACITY AND DISRUPTED EXCITATION-CONTRACTION COUPLING

Van Helden, Ruben - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
de Korte, Tessa - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Kostidis, Sarantos - *Center for Metabolomics & Proteomics, Leiden University Medical Centre, Leiden, Netherlands*

Mei, Hailiang - *Moleculaire Epidemiologie, Leiden University Medical Centre, Leiden, Netherlands*
Slieker, Roderick - *Cell and Chemical Biology, Leiden University Medical Centre, Leiden, Netherlands*
Knoops, Kevin - *Multimodal Molecular Imaging Institute, Maastricht University, Maastricht, Netherlands*
Mulder, Aat - *Cell and Chemical Biology, Leiden University Medical Centre, Leiden, Netherlands*
Jost, Carolina - *Cell & Chemical Biology, Leiden University Medical Centre, Leiden, Netherlands*
Ward-van oostwaard, Dorien - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*
de Coo, René - *Toxicogenomics, Maastricht University, Leiden, Netherlands*
Mummery, Christine - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*
Bellin, Milena - *Anatomy & Embryology, Leiden University Medical Centre, Leiden, Netherlands*

Abstract: Infantile cardiomyopathy is a rare disease associated with severe mitochondrial dysfunction and death shortly after birth. We derived human induced pluripotent stem cells (hiPSCs) from two sibling patients and their healthy mother. The patients were diagnosed with the autosomal recessive disorder Combined Oxidative Phosphorylation Deficiency 8 and carried three compound mutations in the nuclear-encoded mt-Alanine-tRNA synthetase 2 gene (AARS2). We used CRISPR/cas9 to correct the maternally-inherited mutation to create isogenic lines. In the patient hiPSC-derived cardiomyocytes (hiPSC-CMs), we found lower oxidative phosphorylation (OXPHOS) and glycolysis rates, reducing overall ATP generation. Strikingly, there was a 30-60% increase in mitochondrial copy number, and the mitochondrial membrane potential was increased compared to isogenic controls. Expression of the AARS2 protein and the OXPHOS complex 2 peptide MTCO1 was decreased, while the other OXPHOS complexes remained unchanged. Quantitative metabolomics in tandem with RNA-sequencing revealed that patient hiPSC-CMs used phospholipids and amino acids as alternative sources of pyruvate to compensate for the lack of ATP or NAD+. Further KEGG and GO analysis showed altered gene expression in pathways related to phosphorylation, kinase activity, cardiomyopathy, and calcium- and ion-channel expression. To complement gene expression with functional read-outs, we measured intracellular calcium dynamics. Patient hiPSC-CMs had slower beating rates and altered calcium dynamics but, more importantly, a higher incidence of arrhythmic behaviour. Arrhythmia was also evident during contraction under paced conditions at 1 Hz and higher. Finally, microelectrode array measurements showed shortened field potential duration in the patient hiPSC-CMs and reduced sensitivity to E4031 and Flecanaide, which was confirmed with calcium measurements. In sum, we showed that hiPSC-CMs carrying mutations in AARS2 had a lower metabolic capacity, dysfunctional calcium handling and disrupted excitation-contraction coupling, capturing features of the cardiomyopathy observed in patients.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729). The Netherlands Organ-on-Chip Initiative is an NWO Gravitation project (024.003.001)
Keywords: hiPSC-derived cardiomyocytes, Mitochondria and metabolism, disease modelling

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CRISPR-CORRECTION RESCUED SEVERE HYPERTROPHY IN IPSC-DERIVED CARDIOMYOCYTES FROM LZTR1-ASSOCIATED NOONAN SYNDROME PATIENT

Busley, Alexandra - *Cardiology & Pneumology, University Medical Center, Göttingen, Germany*
Böhmer, Linda Berit - *Cardiology & Pneumology/ Stem Cell Unit, University Medical Center Göttingen, Germany*
Kleinsorge, Mandy - *German Federal Institute for Risk Assessment, German Federal Institute for Risk Assessment, Berlin, Germany*
Wollnik, Bernd - *Human Genetics, Institut for Human Genetics, Göttingen, Germany*
Hammer, Elke - *Department of Functional Genomics, University of Greifswald, Germany*
Cyganek, Lukas - *Cardiology & Pneumology/ Stem Cell Unit, University Medical Center Göttingen, Germany*

Abstract: Noonan syndrome (NS) is a multisystem disorder with a broad spectrum of clinical symptoms and varying degrees of disease severity. NS comes along with a quite high prevalence of 1 in 1,000 to 2,500 live births. Common symptoms are low-set ears, dysmorphic facial appearance, a webbed neck, chest deformities and in many cases a severe form of congenital heart disease, e.g. hypertrophic cardiomyopathy (HCM). NS belongs to the spectrum of RASopathies and is causally linked to mutations in a number of genes including PTPN11, SOS1, RAF1 and RIT1, leading to an increased RAS/MAPK signaling pathway activity. In addition, dominant as well as recessive mutations in a newly NS-associated gene called LZTR1 were discovered. As the pathomechanisms behind the manifestation of NS-linked HCM and especially the role of LZTR1 is not entirely understood, we investigated patient-derived induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) from a NS patient harbouring the homozygous variant LZTR1L580P. Further, CRISPR/Cas9 technology was applied to correct the respective gene variant. Proteomic profiling of the patient-derived and CRISPR-corrected iPSC-CMs revealed increased levels of RAS/MAPK- and HCM-associated candidates in diseased cells, such as accumulation of LZTR1-associated interaction partners RIT1 and RAS, indicating a non-functional degradation of these RAS isoforms by the LZTR1-CUL3 complex in the heart. In line, patients' iPSC-CMs displayed cellular hypertrophy that could be rescued upon genetic correction. Summarizing, our iPSC-CM model consisting of a NS patient carrying a recessive LZTR1 mutation could recapitulate major hallmarks of the disease and is a suitable tool to uncover patient-specific disease pathomechanisms as well as to identify personalized therapeutic strategies for NS and NS-associated HCM.

Keywords: Noonan syndrome, CRISPR/Cas9 genome-editing, iPSC-dervied cardiomyocytes

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THOROUGH VALIDATION OF A 3D IN VITRO HUMAN ECTODERMAL ORGANOID MODEL REVEALS HIGH RESEMBLANCE TO IN VIVO NEURAL CREST DEVELOPMENT

Perera, Surangi N. - *Neural Crest Development and Disease Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*
Hsin, Jenny - *Neural Crest Development and Disease Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*
Kerosuo, Laura - *Neural Crest Development and Disease Unit, Neural Crest Development and Disease Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*
Mostrom, Janet - *Neural Crest Development and Disease Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA*

Abstract: The neural crest (NC) is a unique transient stem cell population in the vertebrate embryo that originates from the neuroectoderm and gives rise to a myriad of cell types, including neurons and glia of the peripheral nervous system, facial cartilage and bone, pigment cells of the skin and secretory cells of the endocrine system. Abnormalities in the generation, migration, and differentiation of NC cells results in a range of pathologies termed neurocristopathies. To fully understand and address neurocristopathies, we need a better understanding of human NC development. It is difficult, if not impossible, to study human NC cells in the embryo as they develop at very early stages, however, human NC cells can be derived from pluripotent stem cells in culture. While several existing approaches enable derivation of NC cells, the developmental process the cells undergo under these different in vitro conditions is poorly understood. Here, we characterize a 3D in vitro ectodermal organoid model used to generate NC cells from pluripotent cells step by step with the aim of discovering molecular details of human NC formation. By using bulk and single cell RNA sequencing combined with immunohistochemistry and fluorescent in situ hybridization we compared the transcriptome of our samples from a 10 day 3D organoid process to those generated from in vivo derived NC cells in other animal models and found astonishingly high chronological and molecular level resemblance. Interspecies comparison reveals the ectodermal organoids initially express known early neural plate border markers, followed by early NC specifier genes accompanied by markers driving epithelial to mesenchymal transition, and finally the cells migrating out from the organoids recapitulate a mesenchymal NC signature as seen in vivo in the chick embryo and other animal models. Furthermore, we report that extended cultures of the organoids prolongs the generation of migratory NC cells in a posteriorizing fashion; while the first stream is equivalent to the anterior axial level, the last stream presents a Hox gene signature similar to the trunk NC. Overall, we provide detailed information on the developmental process of human NC cells and an avenue to study a wide range of neurocristopathies that arise due to the defects in the various developmental stages of NC cells.

Keywords: Neural crest cells, Ectodermal organoid, Development

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MECHANICAL SIGNALING UNDERLIES PATTERN FORMATION OF MESODERM IN HESC COLONIES THROUGH FORCE-DEPENDENT BETA-CATENIN REGULATION

Ayad, Nadia M E - *Bioengineering, University of California, San Francisco, CA, USA*
Lakins, Johnathon - *Center for Bioengineering and Tissue Regeneration, Department of Surgery, University of California, San Francisco, CA, USA*
Weaver, Valerie - *Department of Anatomy, Department of Bioengineering and Therapeutic Sciences, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA*

Abstract: Control of cell fate and recapitulation of tissue organization has been a longstanding goal of tissue engineering. While the role of genetics and soluble morphogens has been heavily studied, how mechanical cues affect the regulation of cell differentiation at a molecular level has been an area of growing interest. To probe the contribution of mechanical cues, we have used human embryonic stem cells (hESCs) on gels of defined stiffness as a model of gastrulation-stage embryos. On the periphery of geometrically confined hESC colonies cultured on soft substrates, we observed elevated cellular contractility through traction force microscopy in discrete regions that preceded emerging primitive streak-like features. On those areas, cells express T (Brachyury) and undergo epithelial-to-mesenchymal transition, markers for commitment to a mesoderm progenitor fate. We could ectopically increase forces by localized stretching or decrease local stresses by using different geometries, showing a correlated ectopic increase or decrease of expression of T, respectively, emphasizing a critical role for tissue architecture in early development. Regulating the extent of traction forces exerted by cells with different actomyosin regulators also affected the signaling dynamics of this system. Rho kinase inhibition decreased stresses and subsequently delayed and decreased T expression; conversely, by inducing actin polymerization and stabilizing actin with Jasplakinolide, we could increase traction forces and later assess faster and increased T expression. To further understand the molecular role of elevated tension, we saw that high forces promoted the release of beta-catenin from adhesion junctions and further Wnt signaling through a force-dependent conformational exposure of the tyrosine 654 (Y654) of cadherin-bound beta-catenin. By using a dephosphomimetic mutant for beta-catenin (Y654F), we showed that expression of T is severely affected, indicating a mechanism whereby high cell-cell tension initiates and spatially restrict a release of beta-catenin from junctions, driving Wnt signaling to promote further mesoderm specification. Our work provides a framework to understand how mechanical cues regulate the self-organization of tissues in developmental processes, such as gastrulation.

Keywords: mechanobiology, morphogenesis, gastrulation

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BLASTOCYST-LIKE STRUCTURES GENERATED FROM HUMAN PLURIPOTENT STEM CELLS

Yu, Leqian - *Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA*
Wei, Yulei - *Molecular Biology, UT southwestern, Dallas, TX, USA*
Schmitz, Daniel - *Molecular Biology, UT Southwestern, Dallas, TX, USA*
Sakurai, Masahiro - *Molecular Biology, UT Southwestern, Dallas, TX, USA*
Wu, Jun - *Molecular Biology, UT Southwestern, Dallas, TX, USA*

Abstract: Limited access to embryos has hampered the study of human embryogenesis and disorders that occur during early pregnancy. Human pluripotent stem cells provide an alternative means to study human development in a dish. Recent advances in partial embryo models derived from human pluripotent stem cells have enabled human development to be examined at early post-implantation stages. However, models of the pre-implantation human blastocyst are lacking. Starting from naive human pluripotent stem cells, here we developed an effective three-dimensional culture strategy with successive lineage differentiation and self-organization to generate blastocyst-like structures in vitro. These structures—which we term 'human blastoids'—resemble human blastocysts in terms of their morphology, size, cell number, and composition and allocation of different cell lineages. Single-cell RNA-sequencing analyses also reveal the transcriptomic similarity of blastoids to blastocysts. Human blastoids provide a readily accessible, scalable, versatile and perturbable alternative to blastocysts for studying early human development, understanding early pregnancy loss and gaining insights into early developmental defects.

Keywords: Blastocsyt-like structure, Human Naive Pluripotent Stem Cells, Embryo model

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

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MODULAR 3D NVU-ON-A-CHIP MODEL FOR STUDYING VASCULAR DEMENTIA

Nahon, Dennis - *Anatomy and Embryology, Leiden University Medical Centre, Den Haag, Netherlands*
Frimat, Jean Philippe - *Human Genetics, Leiden University Medical Center, Leiden, Netherlands*
Hu, Michel - *Human Genetics, Leiden University Medical Center, Leiden, Netherlands*
Mummery, Christine - *Anatomy & Embryology, Leiden University Medical Center, Leiden, Netherlands*
Orlova, Valeria - *Anatomy & Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Neurodegenerative diseases are an increasing burden on society. Accumulating evidence indicates a fundamental role of the dysfunction of the brain vasculature in these pathophysiological conditions, resulting in vascular dementia. We have developed a 3D multicellular model that enables investigation of the complex interplay between the multiple cell types composing the neurovascular unit (NVU). Microvascular networks were formed and quantified in triple cultures of human induced pluripotent stem cell-derived ECs (hiPSC-ECs), vascular smooth muscle cells (vSMCs) or primary human brain vascular pericytes (HBVPs), and primary or iPSC-derived astrocytes. Unexpectedly, the ability to support 3D vessel formation by mature iPSC-derived astrocytes was reduced when compared to primary astrocytes. We hypothesised that this is likely a result of the prolonged 2D in vitro culture that renders them in a more reactive state. We further show that rather immature iPSC-derived astrocytes differentiated using a protocol that results in a mixture of astrocytes and neurons showed improved ability to support the development of a perfusable 3D vascular network. Examination of the detailed structural architecture using confocal microscopy showed that the cells oriented themselves similarly as found in vivo. In conclusion, we were able to robustly generate a 3D NVU-on-a-chip model comprised of hiPSC-derived cells. Future work using patient derived hiPSCs will enable us to model the vascular aspects of neurodegenerative diseases.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729). The Netherlands Organ-on-Chip Initiative is an NWO Gravitation project (024.003.001).
Keywords: Organ-on-Chip, Neurovascular unit, Astrocytes

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DMD MOUSE MODEL DISEASE SEVERITY AFFECTS HSMPC SYSTEMIC DELIVERY

Skuratovsky, Emily J. - *MIMG, University of California, Los Angeles, North Hollywood, CA, USA*

Abstract: 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 long-term 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. Here we show that human SMPCs can be delivered to multiple muscles, but after evaluation we observed that in DMD mouse models mdx and mdxD2, a more severe model, 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 muscle cell heterogeneity between healthy, dystrophic, and severely dystrophic muscles. Moreover, we identified differentially regulated genes in mdxD2-NSG capillary endothelial cells (ECs) compared to wt-NSG and mdx-NSG muscle ECs, revealing EC functional impairment in mdxD2-NSG.

Keywords: Endothelial Cells, Mouse Models, Duchenne Muscular Dystrophy (DMD)

TOPIC: EPITHELIAL_GUT

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MODELING NEUROENDOCRINE NEOPLASMS IN HUMAN GASTROINTESTINAL ORGANOIDS

Martinez-Silgado, Adriana - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Wester, Roel - *Department of Molecular Biology, Radboud University, Nijmegen, Netherlands*

Beumer, Joep - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Geurts, Maarten - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Begthel, Harry - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Korving, Jeroen - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Bannier, Marie - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*
Dayton, Talya - *Lab of Hans Clevers, Hubrecht Institute, Utrecht, Netherlands*

Abstract: Hormone-producing neuroendocrine neoplasms (NENs) comprise a set of rare tumors harboring a wide range of genetic alterations. NENs arise in epithelia containing endocrine cells, including the pancreas and the gastrointestinal (GI) tract. Currently, there is no model to study how the different genetic alterations contribute to disease progression. Therefore, we combined human small intestinal organoids and CRISPR-Cas9 base editing to probe the role of genes commonly mutated in GI-NENs, including CDKN1B, MEN1, EPAS1, NF1, IPMK, RB1, and TP53. We found that MEN1 loss of function expands the population of endocrine cells in organoids. Using RNA sequencing we identified differences in the expression of proliferation markers and HOX genes. This altered HOX gene expression pattern could suggest an involvement of the epigenetic machinery. To test this, we are currently analyzing the effect of MEN1 loss on histone modifications in this context. Next, we will perform targeted drug-sensitivity assays in the mutant organoids. In conclusion, our GI-NENs organoid models can contribute to understanding both the mechanisms that drive NEN initiation and potential vulnerabilities that can be exploited for therapeutic purposes.

Funding Source: NETRF: Neuroendocrine Tumor Research Foundation
Keywords: Intestinal organoids, neuroendocrine neoplasms, enteroendocrine cells

TOPIC: EPITHELIAL_LUNG

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VAPING E-LIQUIDS MEDIATE CYTOTOXICITY AND DYSREGULATE HEALTH AND FUNCTION OF HUMAN IPSC-DERIVED LUNG EPITHELIAL CELLS IN A SEX-DEPENDENT MANNER

Ho, Miriel - *Stem Cells, CReATe Fertility Centre, Toronto, ON, Canada*
Ho, Mirabelle - *Stem Cells, CReATe Fertility Centre, Toronto, ON, Canada*
Librach, Clifford - *Fertility, CReATe Fertility Centre, Toronto, ON, Canada*

Abstract: The popularity of electronic cigarette use by young pregnant women has risen to similar levels as conventional cigarette smoking, owing to the misconception that it is safer. There is a paucity of studies detailing the effect of prenatal exposure to vaping e-liquids, with only a few describing how aerosolized vapors alter rodent lung function. This underscores the urgency to elucidate the impact of vaping e-liquids on human fetal lung development. Human induced pluripotent stem cell (iPSC) derivatives are phenotypically immature. Here, we established air-liquid interface lung epithelial models to interrogate their health and function after e-liquid exposure. Male and female iPSC lines were differentiated into proximal airway and distal alveolar epithelial cells (all EPCAM+). Heated e-liquids were introduced to epithelial cultures twice daily for three weeks; untreated controls were performed in parallel. Upon e-liquids exposure, we detected a rapid pro-inflammatory response (IL-8, IFN-g) and cytotoxicity of airway and alveolar cultures. These findings were more pronounced in the male alveolar cells which showed elevated levels of apoptosis and caspase-3/7 and -9 activation. E-liquids containing nicotine induced greater cell stress, reflected by augmented generation and mitochondrial accumulation of reactive oxygen species. All e-liquids triggered variable loss of airway and alveolar barrier function (measured by FITC-dextran), especially in female cells. This was supported by attenuated expression of adherens and tight junction proteins (E-cadherin, ZO-1). Gene analyses revealed widespread changes in mucociliary and surfactant transcripts (FOXJ1, MUC5AC, SFTPD); as well as ACE2 and TMPRSS2 expression – mediators of cellular entry for novel coronavirus SARS-CoV-2. Collectively, we demonstrated that vaping e-liquids exert pleiotropic detrimental effects that dysregulate proximal and distal airways in a sex-dependent manner. Notably, compromises in barrier integrity may increase fetal vulnerability to maternally transmitted pathogenic infection. In future, our ex vivo model of early human fetal lung development presents a valuable opportunity to provide insight into the consequences of chronic vaping, including the examination of cellular crosstalk between niche cell types.

Keywords: lung development, vaping, iPSC

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OPTIMIZED ORGANOID CULTURE MEDIA FOR THE LONG-TERM EXPANSION AND EFFICIENT DIFFERENTIATION OF ALVEOLAR EPITHELIAL CELLS

Finn, Johanna - *Research and Development, STEMCELL Technologies, Vancouver, BC, Canada*
Bowden, Catherine - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Brown, Tyler - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Chang, Wing - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Eaves, Allen - *Corporate, STEMCELL Technologies Inc., Vancouver, BC, Canada, Terry Fox Laboratory, BC Cancer, Vancouver, BC, Canada*
Ho, Victor - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Hou, Juan - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Kramer, Philipp - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Louis, Sharon - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Pena, Uriel - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Ronaghan, Natalie - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Rowbotham, David - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Tellis, Marisa - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*
Yoshikawa, Arisa - *Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada*

Abstract: Organoids are emerging as an excellent tool for studying human development and disease. The COVID-19 pandemic has highlighted the importance of physiologically relevant alveolar models that include both alveolar epithelial type 1 (AT1) and type 2 (AT2) cells. To address the need for an alveolar organoid culture system for respiratory research, we developed the PneumaCult™ Alveolar Organoid Expansion and Differentiation Media for the highly efficient expansion of primary human AT2 cells and subsequent differentiation into AT1 cells. To establish alveolar organoids, purified human AT2 cells were seeded in Corning® Matrigel® domes with serum-free PneumaCult™ Alveolar Organoid Expansion Medium; 10 - 14 days later, organoids with spheroid morphology were generated. These organoids contain self-renewing AT2 cells, as indicated by the expression of the AT2-specific markers Pro-SPC and HT2-280 (n = 5 donors). Organoids maintained in Expansion Medium underwent a ≥ 10,000-fold increase in cell numbers within 6 passages, and could often be maintained beyond 10 passages. After expansion, AT2 organoids can differentiate into AT1 cells using the PneumaCult™ Alveolar Organoid Differentiation Medium. Alveolar organoids differentiated for 10 days downregulated the expression of AT2 markers HT2-280 and Pro-SPC, and increased their expression of the AT1 markers HT1-56, RAGE, and GPRC5a, as detected by flow cytometry or immunofluorescence (n = 5). Furthermore, we detected the expression of SARS-CoV-2 entry receptor ACE2, which is present in both undifferentiated and differentiated alveolar organoids. In summary, the PneumaCult™ Alveolar Organoid Expansion and Differentiation Media are highly efficient and reproducible tools for the feeder-free, long-term expansion of AT2 cells and robust differentiation into AT1 cells, which can be used for disease modeling.

Keywords: Alveolar, Organoid, Media

TOPIC: EPITHELIAL_SKIN

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PREFERENTIAL STIMULATION OF MELANOCYTES BY M2 MACROPHAGES TO PRODUCE MELANIN THROUGH VASCULAR ENDOTHELIAL GROWTH FACTOR

Han, Heeju - *College of Medicine, The Catholic University of Korea, Seoul, Korea*
Ju, Ji Hyeon - *Internal Medicine, The Catholic University of Korea, Seoul, Korea*

Abstract: Post-inflammatory hyperpigmentation is a skin discoloration process that occurs following an inflammatory response or wound. As the skin begins to heal, macrophages first exhibit a proinflammatory phenotype (M1) during the early stages of tissue repair and then transition to a pro-healing, anti-inflammatory phenotype (M2) in later stages. During this process, M1 macrophages remove invading bacteria and M2 macrophages remodel surrounding tissue; however, the relationship between macrophages and pigmentation is unclear. In this study, we examined the effect of macrophages on melanin pigmentation using human induced pluripotent stem cells. Functional melanocytes were differentiated from human induced pluripotent stem cells and named as hiMels. The generated hiMels were then individually cocultured with M1 and M2 macrophages. Melanin synthesis decreased in hiMels cocultured with M1 macrophages but significantly increased in hiMels cocultured with M2 macrophages. Moreover, the expression of interleukin-6 and vascular endothelial growth factor was increased in M1 and M2 cocultured media, respectively. Our findings suggest that M2 macrophages, and not M1 macrophages, induce hyperpigmentation in scarred areas of the skin during tissue repair.

Keywords: Melanocyte, Macrophage, vascular endothelial growth factor

TOPIC: EYE AND RETINA

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HIGH THROUGHPUT SCREEN IDENTIFIES DRUGS THAT RESCUE AMD PHENOTYPE IN IPSC DERIVED RPE MODEL OF MACULAR DEGENERATION

Sharma, Ruchi - *NEI, NEI/NIH, Bethesda, MD, USA*
Bharti, Kapil - *NIH, NEI, Bethesda, MD, USA*
Bose, Devika - *NIH, NEI, Bethesda, MD, USA*
Ortolan, Davide - *NIH, NEI, Bethesda, MD, USA*
Nimmagadda, Malika - *University of Wisconsin, USA*

Abstract: Age-related macular degeneration (AMD) is one of the leading causes of vision loss. The disease initiation arises from the retinal pigment epithelium (RPE), a post-mitotic layer of polarized cells that supports photoreceptors and choroidal capillaries. RPE atrophy leads to photoreceptor cell death and capillary loss, and its dysfunction causes the sub-RPE lipoprotein-rich drusen deposits. GWAS studies present evidence of the role of the alternate complement pathway in AMD pathogenesis, but the mechanism of disease initiation and its progression is not unknown. We developed an AMD model using iPSC-RPE to study molecular events triggered by activating alternate complement pathways through C5a and C3a. We discover drugs that rescue alternate complement-induced AMD phenotype in iPSC-RPE, providing potential pharmaceutical intervention for the disease. iPSC lines from subjects of different AMD genetics were differentiated into RPE and were characterized for their polarity (Ezrin, COLIV), junctional integrity (TER) and functionality (phagocytosis). 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. Immunostaining (IF) was used to check APOE deposits, lipid deposits (Nile red), epithelial phenotype (Vimentin). Pathways downstream of complement were discovered with RNA sequencing and corroborated by IF and WB. High throughput screen on 1280 drugs was performed to discover potential drugs that can rescue RPE cell death.CC-HS elicited APOE and lipid deposits, eroded the epithelial phenotype, reduced TER and compromised phagocytic ability of iPSC-RPE. RNA seq revealed the activation of NF-kb and downregulation of autophagy pathways. We confirmed these results by analyzing levels of p65, LC3, ATG5 in iPSC-RPE cells. Drugs were discovered using the HTS, L-745,870, a dopamine receptor antagonist, and aminocaproic acid (ACA), a protease inhibitor, restored CC-HS induced reduced autophagy and suppressed NF-kB activation in addition to reducing lipid deposits and restoring the functionality in iPSC-RPE caused by CC-HS treatment. The AMD model helped us in dissecting out the cell autonomous role of RPE in disease pathogenesis and provides a platform to study the genotype-phenotype relation in AMD.

Keywords: AMD, iPSC-RPE, drug screen, autophagy, Drug screen, disease modeling, nf-kb, APOE, lipid deposits

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STANDARDIZED FABRICATION OF MURINE TESTICULAR ORGANOIDS WITH IMPROVED GERM CELL SURVIVAL

Richer, Guillaume - *Medicine & Pharmacy, Vrije Universiteit Brussel, Jette, Belgium*
Baert, Yoni - *Medicine & Pharmacy, Vrije Universiteit Brussel, Jette, Belgium*
Goossens, Ellen - *Medicine & Pharmacy, Vrije Univeristeit Brussel, Jette, Belgium*

Abstract: Organ cultures have traditionally been used for in vitro spermatogenesis (IVS) in rodents because they best preserve the testicular architecture which is pivotal in achieving IVS. However, they do not offer the ability to access and manipulate single cells, making it an inefficient model for mechanistic studies. Organoids made from testicular cell suspensions offer these features. Although testicular organoid (TO) cultures can result in tissue surrogates with testicular architecture, histological heterogeneity between individual TOs limits reproducibility of the results, offering unreliable readouts. Moreover, germ cell loss is characteristic during the reorganization phase. To fill these gaps, we propose a new TO growth platform: first, a microwell array was tested for the upscale fabrication of TOs from C57BL/6J mice (P5). Then, the focus was put on improving germ cell survival during the first 2 weeks of reorganization of testicular cells. For this, alpha-MEM-based medium, previously found to support TO generation in mice (medium A), was compared to 3 different culture media (B-D) supplemented with other combinations or concentrations of growth factors (n=105 TOs/condition). Finally, different cell seeding densities (I-V) were tested for their ability to recreate consistent histological resemblance to native tissue in the selected culture media (n=105 TOs/condition). In our platform, testicular cells self-assembled into organoids consisting of 1 tubule-like structure and surrounding interstitium. Interestingly, media B and D resulted in the highest amount of germ cells ($p< 0.0001$), comparable to fresh controls. Particularly TOs cultured in medium D also exhibited the largest surface area, indicative for better growth. Finally, TOs cultured in medium D had the best histology when grown at cell density IV and V ($p< 0.05$). Next, candidate factors will be tested in their ability to efficiently differentiate germ cells through meiotic blockage, often observed in IVS cultures. The opportunities TOs offer to manipulate cells are essential for the study of male infertility and the search for potential therapies. Moreover, they permit high-throughput screening of chemicals, thereby substantially reducing the number of animals for the high demanding reproductive toxicity and drug discovery studies.

Funding Source: Y.B. is a postdoctoral fellow of the Research Foundation – Flanders (FWO, 62930)
Keywords: Testicular organoid, spermatogonial stem cells, in-vitro spermatogenesis

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DO ARTERY CELLS GIVE RISE TO BLOOD-FORMING STEM CELLS DURING EMBRYONIC DEVELOPMENT?

Fowler, Jonas L. - *Stem Cell Biology and Regenerative Medicine, Stanford University, Menlo Park, CA, USA*
Zheng, Sherry - *Developmental Biology, Stanford University, Stanford, CA, USA*
Chai, Tim - *Cancer Biology, Stanford University, Stanford, CA, USA*
Nichane, Massimo - *Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Nguyen, Alana - *Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Ang, Lay Teng - *Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA*
Loh, Kyle - *Developmental Biology, Stanford University, Stanford, CA, USA*

Abstract: The specific developmental precursors that give rise to blood-forming hematopoietic stem cells (HSCs) have yet to be fully resolved. Multiple precursors to HSCs have been proposed with the leading hypothesis positing that a subset of endothelial cells give rise to HSCs. Here we employ genetic lineage-tracing to rigorously demonstrate that arterial endothelial cells give rise to HSCs in vivo. Using a Cx40-CreER mouse model, we genetically labeled the first arterial cells that emerge in the developing embryo 8.5 days post conception and found that these cells give rise to HSCs later in development. Applying this knowledge in vitro, we developed a method to efficiently differentiate human pluripotent stem cells (hPSCs) into >90% pure artery cells. These hPSC-derived artery cells could further differentiate into RUNX1+ hemogenic endothelium, and subsequently >90% pure HSC-like cells that could further generate lymphoid, myeloid, and erythroid cells in vitro. Our work identifies artery cells as the specific in vivo precursor to HSCs, advances the generation of HSC-like cells in vitro, and raises the question of why arteries (as opposed to other endothelial cells) are uniquely competent to generate HSCs.

Keywords: Hematopoiesis, Lineage-tracing, Differentiation

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A CHEMICAL INHIBITOR OF NR4A1 ALTERS ASXL1-MUTANT BLOOD STEM CELL CLONAL DYNAMICS

Avagyan, Serine - *Pediatric Hematology/Oncology, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA, USA*
Zon, Leonard - *Pediatric hematology/oncology, Boston Children's Hospital, Boston, MA, USA*

Abstract: Clonal expansion of hematopoietic stem and progenitor cells (HSPCs) with acquired mutations underlies clonal hematopoiesis (CH). Abating this expansion may reduce hematologic and cardiovascular risks associated with CH. Here, we demonstrate a chemical that can alter mutant clonal dynamics in vivo. We recently published a zebrafish model to study CH, called TWISTR, combining mosaic CRISPR-Cas9 mutagenesis with color labeling of HSPC clones. We identified the critical role of inflammation in promoting the fitness of mutant clones. With heightened inflam-

matory state in the marrow, mutant progenitors had upregulation of anti-inflammatory genes, including nr4a1, as a means of resistance to inflammation. Using CH with asxl1 mutations, we showed that genetic abrogation of nr4a1 prevented the expansion asxl1 clones. Here, we tested the effect of pharmacologic inhibition of nr4a1 in adult zebrafish with asxl1-mutant CH. Zebrafish were treated with the inhibitor with a 5-days-on-2-days-off schedule, and clonal dynamics was assessed by peripheral blood sampling. In vehicle-treated zebrafish, asxl1 clones with frameshift insertion-deletions (indels) expanded with an average clone size increase of +59.4% [-69.3% to +795.9%] from baseline after 3 months ($p < 0.003$, Wilcoxon rank test). Inhibition of nr4a1 was associated with significantly reduced, non-significant expansion of asxl1-mutant clones with frameshift indels, with an average increase of +13.4% [-91.1% to +391.9%]. No significant changes were observed in clones with in-frame indels in either cohort. Examination of mutant clonal dynamics revealed several important findings. In vehicle-treated mutant zebrafish, small clones of < 10% were more likely to expand than large clones of >10% (+106.3% vs +5.8%, $p < 0.02$). Clones with in-frame indels largely remained constant. However, in zebrafish with a significant growth of a clone with a frameshift indel, we observed a concurrent diminishing of another clone with an in-frame indel. This suggested an interclonal regulation of HSPC clone size in CH. Mutant clones in the experimental cohort halted their growth but did not diminish with treatment. We successfully used TWISTR to study the clonal dynamics of asxl1 induced CH, and showed that pharmacologic inhibition of a pro-survival pathway could be used for the treatment of CH.

Keywords: clonal hematopoiesis, hematopoietic stem cell, zebrafish

TOPIC: IMMUNE SYSTEM

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IN VIVO AND IN VITRO GENERATION OF PATIENT SPECIFIC THYMIC EPITHELIAL CELLS

Ramos, Stephan A. - *Pediatrics, University of Colorado-Barbara Davis Center, Sunnyvale, CO, USA*
Jimeno, Antonio - *Medicine, University of Colorado, Aurora, CO, USA*
Kappler, John - *Immunology and Microbiology, University of Colorado, Aurora, CO, USA*
Morton, John - *Medicine, University of Colorado, Aurora, CO, USA*
Reed, Brendan - *Immunology and Microbiology, University of Colorado, Aurora, CO, USA*
Russ, Holger - *Pediatrics, University of Colorado, Aurora, CO, USA*

Abstract: The thymus is a glandular organ that is necessary for the development of the T cell repertoire. Until recently, studies on thymus development and autoimmunity have been largely restricted to mouse models, due to the lack of a faithful human thymus models. We have established a universal differentiation approach that can derive thymic epithelial progenitor cells (TEPs) from multiple patient specific induced pluripotent stem cell (iPSCs) lines. Transplanted TEPs further differentiate into functional thymic epithelial cells (TECs) after months in vivo. Human TECs have the ability to educate developing mouse T-cells and are indistinguishable from primary neonatal TECs by scRNAseq analysis, indicating the generation of a relevant and mature TEC phenotype. By co-culturing of iPSC derived TEPs with hemopoietic stem cells we established stem cell derived thymic organotypic cultures (sTOs) in vitro. sTOs are capable of recapitulating aspects of human thymus development, support TEC differentiation and potentially T cell development within weeks. Notably sTOs exhibit expression of critical regulators of negative selection, namely AIRE and multiple tissue restricted antigens. Taken together, we anticipate that our results have important implications for current efforts geared towards generating functional thymic and T cells for future cell therapy approaches.

Funding Source: NIH/NIDDK grant R01DK12044 and P30DK116073, NIDDK/HIRN RRID : SCR_014393; UC24 DK1041162, Culshaw Junior Investigator Award in Diabetes, Children's Diabetes Foundation, the Gate's Grubstake Fund.
Keywords: Thymus, Organoids, Tcells

TOPIC: KIDNEY

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TUNING PRECURSOR POPULATIONS IN SYNCHRONIZED NEPHRON-FORMING KIDNEY ORGANOIDS

Lindstrom, Nils O. - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Schnell, Jack - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Achieng, Mary Anne - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Fausto, Connor - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*

Abstract: Cells of the nephron proximal tubule are the principal cells injured and dysregulated in kidney disease. They are not fully replicated in kidney organoid models where cells lack features of maturation and bona fide proximal precursor tubule profiles. Insights from mouse studies show proximal tubule differentiation is dependent on Hepatocyte nuclear factor 4a (Hnf4a) and Notch signaling and patients with mutations in HNF4A and Notch-ligand JAG1 display nephrocalcinosis, Fanconi, and Alagille syndrome indicative of perturbed normal proximal tubule function. Beside highly derived immortalized cell-lines there are no models for human proximal tubule cells that can be used to develop regenerative therapies, identify new drug targets, and provide genetic diagnostics for patient-specific therapeutic plans. Here we focus on establishing a framework for normal proximal tubule development and contrast it to organoid nephron patterning to identify malprogrammed steps in in vitro patterning. We scrutinize in vivo nephrogenesis and show that a small cell plaque in the early human nephron displays a transcriptional and protein signature of putative proximal tubule precursors, providing a spatial coordinate for proximal tubule origins. Computational and anatomical tracing of this cell-population shows a series of developmental transitions from a multi-potent nephron tubular precursor that is gradually refined into the putative proximal precursors. Following this in vivo blueprint for human nephron development, we modify developmental domains in the early kidney organoid by manipulating signaling pathways through the action of small-molecule inhibitors. Tuning early nephron development results in organoid nephrons that phenocopy in vivo development and drives

a JAG1+/HNF1B+ early proximal signature. This cellular domain prefigures a transition to a HNF4A+ late proximal cell-profile with increased expression of HNF4A. Transcriptional profiling of tuned organoids shows a drastic increase in genes imparting proximal tubule physiologies. Shifting organoid nephron-domains therefore alters their maturation potential and provides an important tool to generate cells for high-fidelity modeling of renal disease, screening nephrotoxicity, and developing replacement therapeutics.

Keywords: Kidney organoid, Proximal tubule, Renal Physiology

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BIMATOPROST AMELIORATES COLISTIN INDUCED NEPHROTOXICITY

Lina, Joo - *Microbiology, CHA University/CHA Bio Complex, Bundang-gu, Seongnam-si,*
Jeong, Hye Yun - *Nephrology, CHA University, Bundang-gu, Seongnam-si, Korea*
Jee, JooHyun - *Microbiology, CHA University, Bundang-gu, Seongnam-si, Korea*
Choi, Woo Hee - *Microbiology, CHA University, Bundang-gu, Seongnam-si, Korea*
Bae, Dong Hyuck - *Microbiology, CHA University, Bundang-gu, Seongnam-si, Korea*
Yoo, Jongman - *Microbiology, CHA University, Bundang-gu, Seongnam-si, Korea*

Abstract: Colistin (polymixin E) is an effective antibiotics against multidrug resistant Gram negative bacteria(MDR-GNB). However, the high rates of nephrotoxicity caused by colistin limits its clinical use. To find out a new compound, which can ameliorate colistin induced nephrotoxicity, we obtained 2,485 compounds from korea chemical bank. Among these compounds, we unveiled that bimatoprost (prostaglandin F2α) could ameliorate colistin induced nephrotoxicity using High-content-screening (HCS) image based assay. To investigate whether bimatoprost effectively ameliorates colistin induced nephrotoxicity, the in vitro and in vivo tests were performed. In the model of in vitro cultured human proximal tubular cells (HK-2), colistin induced cell cytotoxicity in dose dependent manner. The number of TUNEL positive cells indicating apoptosis increased in colistin treated group, and decreased in colistin plus bimatoprost group. The reactive oxygen species (ROS) generation measured by DCF-DA decreased in colistin plus bimatoprost group than only colistin treated group. We additionally used kidney organoids derived from human pluripotent stem cells to evaluate the effect of bimatoprost, and it alleviated the colistin-induced proximal tubule cell damage. We used Female C57BL/6 mice for In vivo study, and these (n = 5 in each group) were randomly divided into the four groups for intraperitoneal injection of 14 consecutive days. Normal saline and 1% DMSO were injected for control group, and Colistin 10mg/kg/12hr, Bimatoprost 0.5mg/kg/12hr, or Bimatoprost 0.5mg/kg/12hr plus colistin 10mg/kg/12hr was injected for each group, respectively. Colistin injection increased the serum levels of blood urea nitrogen and creatinine. However these serum levels decreased in bimatoprost injection group. Furthermore, the levels of Tunel positive marker and kim-1(kidney injured marker-1) were lower in bimatoprost treated group. In this study, we present that bimatoprost might be a novel chemical that could ameliorate the colistin induced nephrotoxicity

Funding Source: 3D-TissueChip Based Drug Discovery Platform Program through the Korea Evaluation Institute of Industrial Technology funded by the Ministry of Commerce, Industry and Energy (20009773)
Keywords: HCS(High-content screening), Drug repurposing, Kidney organoid, Nephrotoxicity, Kidney injury, Colistin induced nephrotoxicity, Colistin, antibiotics, novel chemical,

TOPIC: MUSCULOSKELETAL

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USING DEVELOPMENTALLY RELEVANT MECHANISMS TO DESIGN A NOVEL STEM CELL-MODEL FOR NEUROMUSCULAR DISORDERS

Bursalyan, David - *Department of Neurobiology, University of California, Los Angeles, North Hills, CA, USA*
Butler, Samantha - *Department of Neurobiology, University of California, Los Angeles, CA, USA*
Gupta, Sandeep - *Department of Neurobiology, University of California, Los Angeles, CA, USA*

Abstract: Neuromuscular disorders encompass many serious diseases, including Amyotrophic Lateral Sclerosis, Myasthenia gravis, and other muscular dystrophies. These neuromuscular disorders are characterized by the dysfunction of the motor circuits that connect motor neurons to specific muscles through neuromuscular junctions (NMJ), resulting in muscle atrophy and the loss of coordinated movement. To get a better understanding of the phenotypes observed in neuromuscular diseases, a simple in vitro model is required that can recapitulate the endogenous onset of disease. All previous in vitro models of motor circuits have relied upon different sources of muscle and motor neurons, which may not accurately mimic the developmental events that lead to formation of NMJs in vivo. Using the logic of early embryonic development, which gives rise to neuromesodermal progenitors that can differentiate into either muscle or spinal neurons, we have designed a novel method of simultaneously differentiating motor neurons and skeletal muscle cells from embryonic stem cells (ESCs) in 2-dimensional culture system. We have used a ESC reporter line (Hb9::GFP) that expresses GFP specifically in motor neurons to assess whether our in vitro model generates muscle and bona fide motor neurons. This approach has allowed us to use live imaging to monitor the growth of motor axons as they make connections with the adjacent muscle fibers. An immunohistochemical analysis with Tuj1 (neurons) and MF20 (muscles) revealed that these cells self-reorganize to form intact motor circuits. We will now characterize the different cell types produced in our model system and determine circuit activity using calcium imaging, working towards using this in vitro model to study disease phenotypes in a Spinal Muscular Atrophy iPSC line. Together, the development of this model will provide insight into the mechanisms of neuromuscular disorders, and thereby enable the design of novel clinical interventions.

Keywords: Embryonic Stem Cells, Neuromuscular Junctions, In-Vitro Model

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ELUCIDATING GENE REGULATORY NETWORKS UNDERLYING HUMAN SKELETAL DEVELOPMENT BY INTEGRATING SINGLE-CELL DYNAMICS OF CHROMATIN ACCESSIBILITY AND GENE EXPRESSION

Tani, Shoichiro - *Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan*
Okada, Hiroyuki - *Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan*
Seki, Masahide - *Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Chiba, Japan*
Suzuki, Yutaka - *Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Chiba, Japan*
Saito, Taku - *Sensory and Motor System Medicine, Graduate School of Medicine, The University Tokyo, Japan*
Tanaka, Sakae - *Sensory and Motor System Medicine, Graduate School of Medicine, The University of Tokyo, Japan*
Chung, Ung-il - *Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan*
Hojo, Hironori - *Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Japan*
Ohba, Shinsuke - *Department of Cell Biology, Institute of Biomedical Sciences, Nagasaki University, Nagasaki, Japan*

Abstract: Elucidating gene regulatory networks (GRNs) underlying complex biological processes is an essential step in understanding the physiology and pathology of our organs and development. Although human pluripotent stem cells (hPSCs) are an attractive cell source for generating specific cell types, recapitulation of 3D bone formation organized by multiple cell types is challenging. At the ISSCR 2021 annual meeting, we proposed a method to recapitulate human endochondral bone formation at embryonic stages by implanting hPSC-derived sclerotome beneath renal capsules of immunodeficient mice. To elucidate GRNs underlying human skeletal development, we analyzed the hPSC-derived bone tissues by single-cell multiome analysis focusing on chromatin accessibility and gene expression. Multimodal clustering analysis detected 10 clusters representing 4 skeletal cell types: skeletal progenitors, chondrocytes, hypertrophic chondrocytes, and osteoblasts. RNA velocity analysis predicted a bidirectional trajectory from skeletal progenitors to chondrocytes and osteoblasts. Chromatin accessibility and the corresponding gene expression changed dynamically along this trajectory. Integrative analysis of motif enrichment with gene expression (average AUC > 0.5) revealed cell-type-specific combinations of transcriptional regulators, indicating dynamics of GRNs in cell fate specification. Motifs of these key regulators were enriched in cell-type-specific chromatin accessible regions linked with marker gene expressions. By integrating cell-type-specific gene expression, RNA velocity, and chromatin accessibility, we identified a novel transcription factor that constitutes GRNs in osteoblasts. In situ hybridization on hPSC-derived bone tissues revealed co-expression of the identified factor with the osteoblast master regulators (RUNX2 and SP7). A knockdown study of the identified factor with short hairpin RNA revealed significant downregulation of osteogenic marker genes and regulators in human mesenchymal stromal cells (FDR < 0.05). Collectively, we demonstrated the dynamics of GRNs underlying human skeletal development and identified a novel transcription factor constituting the networks in human osteogenesis.

Funding Source: This work is supported by The Nakatomi Foundation, Grant-in-Aid for JSPS Fellows (21J12970), and The Japan Agency for Medical Research and Development (JP21bm0704071).
Keywords: single-cell multiome, human skeletal development, gene regulatory networks

TOPIC: NEURAL

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THERAPEUTIC EFFECTS AND RECOVERY MECHANISM OF ADULT HUMAN NEURAL STEM CELLS IN ISCHEMIC STROKE

Joo, Kyeung Min - *Department of Anatomy and Cell Biologty, Sungkyunkwan University School of Medicine, Suwon, Korea*
Lee, Kyung-Hoon - *Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea*
Kim, Chigu - *Business Development Division, Medinno Inc., Seoul, Korea*
Kim, Chung Kwon - *Department of Anatomy & Cell Biology, Sungkyunkwan University School of Medicine, Suwon, Korea*

Abstract: Ischemic stroke is one of the leading causes of death and long-term disability worldwide. Neural stem cells have emerged as a promising potential for ischemic stroke therapy since they can recover damaged brain and neural cells by differentiating into functional neurons and secreting several beneficial factors compared to conventional therapies. In this study, we investigated the therapeutic effect and recovery mechanism by adult human neural stem cells (ahNSCs) in an ischemic stroke animal model. ahNSCs (5×10^5 cells/head) were transplanted into brain of Middle Cerebral Artery Occlusion (MCAO) model of rats, and the cerebral atrophy volume and neurological function were evaluated. The results showed that ahNSCs reduced atrophy volume of ischemic stroke brain and improved neuro-behavioral deficits for 28 days after transplantation by decreasing apoptosis and restoring damaged neural cells in infarct brain regions. Primary cultured rat cortical neuron cells (SCNs) were exposed by Oxygen-glucose deprivation (OGD) for 1 hour and then treated with conditioned media (CM) of ahNSCs for 24 hours. Treatment of CM in cortical neuron cells was increased cell viability by reducing apoptosis and activated JAK/STAT3 pathway by releasing proteins of ahNSCs. Our data indicated that CM of ahNSCs has significant neuroprotective efficacy under OGD damage conditions in primary cortical neuron cells. This study will be usefully applied for developing new techniques for enhancing the therapeutic effect of stem cell treatment in stroke and several neurological diseases.

Keywords: adult human neural stem cells, stroke, JAK/STAT3 pathway

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AN IN VITRO NEUROGENETICS PLATFORM FOR PRECISION DISEASE MODELING IN THE MOUSE

Cortes, Daniel - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Escudero, Melanie - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Mitra, Arojit - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Austin, Korgan - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Edwards, Alyssa - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
O'Connell, Kristen - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Reinholdt, Laura - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*
Pera, Martin - *JAX Mammalian Genetics, The Jackson Laboratory, Bar Harbor, ME, USA*

Abstract: Experimental platforms based on human pluripotent stem cells are playing an increasing role in the study of the development of the central nervous system, and in unraveling the genetics of brain disorders. However, most neurologic or psychiatric disorders are highly complex, involving the interaction of multiple systems inside and outside of the central nervous system (CNS), and ultimately require study at the organismal level. While the mouse is often the model of choice for genetic analysis of neurological disorders, the introduction of disease mutations into a single inbred strain sometimes fails to yield phenotypes relevant to human disease. Recent studies show that interrogating genetically diverse panels of mice can identify better models of human sensitivity and resistance to candidate disease variants. To make mouse genetic diversity resources more accessible to the research community, we developed an in vitro methodology for modeling multiple stages of central nervous system development using a panel of genetically diverse mouse embryonic stem cell lines. The platform allows investigation of neural specification, neurogenesis, neuronal maturation and electrophysiological function, and axonal repair. In proof of concept studies, we analyzed effects of the loss of function of Dyrk1a, a dosage-sensitive neurodevelopmental gene implicated in both Down Syndrome and severe autism. Chemical knockdown of Dyrk1a demonstrated profound strain differences in the cellular response to the ablation of DYRK1A activity throughout development in vitro. Responsive strains showed in vitro developmental defects consistent with observations in vivo on Dyrk1a knockout mice, and transcriptomic analysis of sensitive and resistant cell strain backgrounds successfully identified key molecular pathways in neural development known to be associated with Dyrk1a haploinsufficiency in vivo. Mouse strain-dependent variations in axonal repair in vitro mirrored strain differences in the recovery from damage to the CNS in vivo. Thus, high throughput, comparative in vitro phenotypic analysis of differentiated cells from human and genetically diverse mouse pluripotent stem cells bearing disease mutations can provide a facile route to identification of optimal mouse strains for precision disease modeling in vivo.

Funding Source: The Jackson Laboratory and NIH Grant U42 OD010921 to LGR
Keywords: neurogenetics, neural development, disease modeling, mouse and human pluripotent stem cell

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A NEW MODEL: IS THE BRAIN FORMED FROM TWO SEPARATE CELLULAR ORIGINS?

Dundes, Carolyn - *Stem Cell Biology & Regenerative Medicine, Stanford University, Menlo Park, CA, USA*
Ahsan, Hadia - *Stem Cell Biology & Regenerative Medicine, Stanford University, Stanford, CA, USA*
Jokhai, Rayyan - *Stem Cell Biology & Regenerative Medicine, Stanford University, Stanford, CA, USA*
Salomon, Rachel - *Stem Cell Biology & Regenerative Medicine, Stanford University, Stanford, CA, USA*
Loh, Kyle - *Stem Cell Biology & Regenerative Medicine, Stanford University, Stanford, CA, USA*

Abstract: The brain is arguably the most complex organ in the human body and yet its embryonic origins—specifically its cellular precursors and the sequence of steps through which they develop into a full organ—are incompletely understood. The prevailing model of brain development suggests there is a single brain precursor population within the ectoderm layer with the broad potential to generate the entire brain (forebrain, midbrain, and hindbrain). By contrast, here we show that there are two separate ectodermal precursors to the brain: one for the forebrain/midbrain and another for the hindbrain. We demonstrate this through in vivo genetic lineage tracing in mouse embryos where we observed separate progenitors to the fore/midbrain and hindbrain. In parallel in vitro experiments differentiating human pluripotent stem cells, we determined that the respective fore/midbrain and hindbrain progenitors cannot interconvert. This work suggests the brain derives from two separate progenitors and we therefore propose that the adult brain is a composite tissue arising from two embryonic origins, with wide-ranging implications for development, differentiation, evolution, and disease.

Keywords: brain development, genetic lineage tracing, neural differentiation

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A CONGENITAL HYDROCEPHALUS CAUSING MUTATION IN TRIM71 RESULTS IN STEM-CELL AND NEURAL-DIFFERENTIATION DEFECTS THROUGH INHIBITING LSD1 MRNA TRANSLATION

Hu, Wenqian - *Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA*
Liu, Qiuying - *Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA*
Novak, Mariah - *Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA*
Pepin, Rachel - *Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA*

Abstract: Congenital hydrocephalus (CH) is a major cause of childhood morbidity. Mono-allelic mutations in Trim71, a conserved stem-cell-specific RNA-binding protein, cause CH, however, the molecular basis for pathogenesis mediated by these mutations remains unknown. Here, using mouse embryonic stem cells as a model, we reveal that the mouse R783H mutation (R796H in human) significantly alters Trim71's mRNA substrate specificity and leads to accelerated stem-cell differentiation and neural lineage commitment. The mutant Trim71, but not the wild-type Trim71, binds Lsd1 (Kdm1a) mRNA and represses its translation.

Specific inhibition of this repression or a slight increase of Lsd1 in the mutant cells alleviates the defects in stem cell differentiation and neural lineage commitment. These results determine a functionally relevant target of the CH-causing Trim71 mutant that can potentially be a therapeutic target and provide molecular mechanistic insights into the pathogenesis of this disease.

Keywords: stem cell differentiation, congenital hydrocephalus, RNA-binding protein

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STUDYING NEURON AND GLIA INTERACTION USING A CO-CULTURE MODEL OF HUMAN IPSC DERIVED INTERNEURONS AND ASTROCYTES

Bruzelius, Andreas - *Experimental Medical Science, Lund University, Lund, Sweden*
Stamouli, Christina-Anastasia - *Experimental Medical Science, Lund University, Lund, Sweden*
Rylander Ottosson, Daniella - *Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Schizophrenia (SCZ) is a prevalent and costly psychiatric disorder affecting a large proportion of individuals, however, the molecular mechanisms involved in the complicated pathology of SCZ remains poorly understood. Current in vitro models have difficulty recapitulating the schizophrenic pathology, mainly due to the heterogeneity of the disorder but also the different cell types involved. Astrocytes from schizophrenic individuals have previously been shown to have a defective differentiation pathway, with deficiencies in synaptic potassium homeostasis which could contribute to a significantly lower firing threshold for neuronal cells resulting in network desynchronization. We here propose a patient-specific co-culture system involving both GABAergic interneurons and astrocytes derived from iPSC that would allow us to study both the astrocytic and interneuron development and function with focus on maturation, electrical activity and gene expression from schizophrenic individuals. This would allow us to study the interplay between neurons and glia and potential deficits present for schizophrenic individuals with hope of identifying molecular targets allowing for new drug or treatment applications.

Funding Source: Swedish Research Council Brain Foundation Jeansson Foundation Royal Physiographical Society MultiPark Strategic Research Area at Lund University
Keywords: Cell Model, Neuropsychiatric, Reprogramming

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SILK SCAFFOLDING DRIVES SELF-ASSEMBLY OF FUNCTIONAL AND MATURE HUMAN BRAIN ORGANOIDS

Sozzi, Edoardo - *Experimental Medical Science, Lund University, Lund, Sweden*
Kajtez, Janko - *Experimental Medical Science, Lund University, Lund, Sweden*
Bruzelius, Andreas - *Experimental Medical Science, Lund University, Lund, Sweden*
Wesseler, Milan - *DTU Health Tech, Technical University of Denmark, Copenhagen, Denmark*
Nilsson, Fredrik - *Experimental Medical Science, Lund University, Lund, Sweden*
Birtele, Marcella - *Experimental Medical Science, Lund University, Lund, Sweden*
Larsen, Niels - *DTU Health Tech, Technical University of Denmark, Copenhagen, Denmark*
Ottosson, Daniella - *Experimental Medical Science, Lund University, Lund, Sweden*
Storm, Petter - *Experimental Medical Science, Lund University, Lund, Sweden*
Parmar, Malin - *Experimental Medical Science, Lund University, Lund, Sweden*
Fiorenzano, Alessandro - *Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Human pluripotent stem cells (hPSCs) are intrinsically able to self-organize into cerebral organoids that mimic features of developing human brain tissue. These three-dimensional structures provide a unique opportunity to generate cytoarchitecture and cell-cell interactions reminiscent of human brain complexity in a dish. However, current in vitro brain organoid methodologies often result in intra-organoid variability, limiting their use in recapitulating later developmental stages as well as in disease modeling and drug discovery. In addition, cell stress and hypoxia resulting from long-term culture lead to incomplete maturation and cell death within the inner core. Here, we used a recombinant silk microfiber network as a scaffold to drive hPSCs to self-arrange into engineered cerebral organoids. Silk scaffolding promoted neuroectoderm formation and reduced heterogeneity of cellular organization within individual organoids. Bulk and single cell transcriptomics confirmed that silk cerebral organoids display more homogeneous and functionally mature neuronal properties than organoids grown in the absence of silk scaffold. Furthermore, oxygen sensing analysis showed that silk scaffolds create more favorable growth and differentiation conditions by facilitating the delivery of oxygen and nutrients. The silk scaffolding strategy appears to reduce intra-organoid variability and enhances self-organization into functionally mature human brain organoids

Keywords: Cerebral organoids, Silk scaffold, Human pluripotent stem cells

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REGIONALLY DEFINED PROTEOMIC PROFILES OF HUMAN CEREBRAL TISSUE AND ORGANOIDS REVEAL CONSERVED MOLECULAR TENANTS OF NEURODEVELOPMENT

Melliou, Sofia - *Laboratory of Medicine and Pathobiology, University of Toronto, ON, Canada*
Sangster, Kevin - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*
Kao, Jennifer - *Physiology, University of Toronto, ON, Canada*
Zarrei, Mehdi - *The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada*
Lam, Brian - *Laboratory of Medicine and Pathobiology, University of Toronto, ON, Canada*
Howe, Jennifer - *The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada*
Papaioannou, Michael - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*
Tsang, Queenie - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*
Abbasi-Borhani, Okty - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*
Sajid, Rifat - *Laboratory of Medicine and Pathobiology, University of Toronto, ON, Canada*
Bonnet, Celine - *Centre hospitalier regional universitaire de*

Nancy, University Hospital Centre Nancy, France
Leheup, Bruno - *Centre hospitalier regional universitaire de Nancy, University Hospital Centre Nancy, France*
Shannon, Patrick - *Laboratory of Medicine and Pathobiology, University of Toronto, ON, Canada*
Scherer, Stephen - *The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada*
Stavropoulos, Dimitri James - *Genome Diagnostics, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada*
Djuric, Ugljesa - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*
Diamandis, Phedias - *Research, Princess Margaret Hospital Cancer Centre, Toronto, ON, Canada*

Abstract: Cerebral organoids have emerged as faithful human-oid avatars for modelling advanced neurodevelopmental and pathological processes. In addition, they hold great potential as tools for probing difficult to study and less understood aspects of the human brain. Towards this latter prospect, we leveraged mass spectrometry-based proteomics to molecularly profile pre-cursor and more committed neural compartments of both hu-man-derived organoids and mid-gestation fetal brain tissue, to define overlapping protein-level programs. Our analysis included precursor-enriched transcriptional regulatory proteins that were notably not found to be differentially expressed in previous tran-scriptomic datasets. To highlight the discovery potential of this resource, we showed that the RuvB-like 2 (RUVBL2) AAA-type ATPase is preferentially expressed in the SOX2-positive com-partment of cerebral organoids and chemical inactivation leads to precursor cell displacement and apoptosis within the more mature DCX-positive niche. We extended our work to explore potential clinicopathological correlates of this cytoarchitectural disruption, by interrogating clinical datasets and identifying rare de novo genetic variants involving RUVBL2 in patients diagnosed with neurodevelopmental impairments. Our study demonstrates how cell-type-specific and phenotype-level profiling of cerebral organoids can help nominate previously unappreciated genes in neurodevelopment. The generalization of this approach provides a promising system that could offer new insights into less acces-sible aspects of human brain biology, including pathogenesis and treatment approaches for neurodevelopmental and neurological disorders.

Keywords: Neurodevelopment., Cerebral Organoids, Mass spectrometry-based proteomics

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PERTURB-SEQ SCREEN IN HUMAN IPSC-DERIVED NEURONS REVEALS GENETIC MODIFIERS OF TUBEROUS SCLEROSIS COMPLEX (TSC)

Yang, Joyce L. - *Advanced Cellular Technologies, Insitro, Palo Alto, CA, USA*
Yang, Joyce - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Mohammadi, Shahin - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Ranadive, Ninad - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Zeng, Haoyang - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Sandakli, Ahmed - *Genomics, Insitro, South San Francisco, CA, USA*
Prasad, Pooja - *Genomics, Insitro, South San Francisco, CA, USA*
Locht, Hans - *Genomics, Insitro, South San Francisco, CA, USA*
Salick, Max - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Pan, Meiliang - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Ranu, Navpreet - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Tu, Shengjiang - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Sharon, Eilon - *Data Science and Machine Learning, Insitro, South San Francisco, CA, USA*
Chu, Ci - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*
Kaykas, Ajamete - *Advanced Cellular Technologies, Insitro, South San Francisco, CA, USA*

Abstract: Tuberous sclerosis complex (TSC) is an autosomal dom-inant genetic disorder characterized by benign tumor growth in organs throughout the body with an estimated 2 million people affected worldwide. It is caused by loss of function mutations in the TSC1 or TSC2 genes that lead to over-activation of the mech-anistic target of rapamycin (mTOR) pathway. Despite advances in understanding the underlying disease biology and regulatory approval of mTOR inhibitors to manage symptoms, there is still an unmet need for a safe and effective treatment across all TSC manifestations. Here, we identified genetic modifiers of TSC2 knockout (KO) phenotype using a Perturb-Seq screen (pooled CRISPR screen with single-cell RNA-seq readout) in human neu-rogenin2 (NGN2) neurons derived from induced pluripotent stem cells (iPSCs) in both the wild-type (WT) and TSC2 KO genetic backgrounds. We used CRISPR-Cas9 to knock out 170 genes im-plicated in TSC disease biology, and performed single-cell RNA sequencing on day 14 NGN2 neurons. Next, we built a regression model for WT and TSC2 KO transcriptional profiles using Orthog-onal Partial Least Squares (O-PLS), a supervised multivariate data projection method. We then projected the transcriptional profiles of each CRISPR KO onto the O-PLS model and ranked genes as modifiers of the transcriptional phenotype. In addition, we used a complementary, unsupervised approach: we built a cell state manifold using ACTIONet and computed the diffusion distance of each CRISPR KO cell from the unperturbed WT and TSC2 KO cells. The two approaches identified the same top ranked modifi-ers. Reassuringly, we also identified TSC1 and TSC2 as the strong-gest disease-inducing genes in the WT background, as well as known disease-reverting genes in the TSC2 KO background. Our work demonstrates that Perturb-Seq coupled with machine learn-ing based transcriptional analysis can be used as an unbiased way to screen for genetic modifiers of disease and to potentially uncover novel therapeutic targets.

Keywords: iPSC-derived NGN2 neurons as TSC disease model, Perturb-Seq screen for genetic modifiers, Machine learning

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PARK7 LOSS OF FUNCTION INDUCES LYSOSOMAL AND PROTEOSTASIS DISRUPTION IN MIDBRAIN ASTROCYTES

Morrone Pafitt, Gustavo - *Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
Coccia, Elena - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Goldman, Camille - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Whitney, Kristen - *Neuroscience, Icahn School of Medicine, New*

York, NY, USA
Reyes, Ricardo - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Sarrafha, Lily - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Crary, John - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Ordureau, Alban - *Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
Ahfeldt, Tim - *Neuroscience, Icahn School of Medicine, New York, NY, USA*
Blanchard, Joel - *Neuroscience, Icahn School of Medicine, New York, NY, USA*

Abstract: Early onset Parkinson's disease (PD) is a severe form of neurodegeneration that affects the midbrain, among other regions, causing neuronal death in susceptible neuronal populations. Non-cell autonomous contribution to dopaminergic cell death in PD remains poorly understood and essential for the full understanding of the pathology progression. Astrocytes, although more resilient to cell death, display damage in PD with deleterious consequences for neuro-astrocytic function. Loss of function variants in DJ1 (PARK7) cause autosomal recessive early onset PD by mechanisms that are not fully understood. Glycation and Advance glycation product (AGES) formation are a deleterious process that erodes cellular function overtime with implications for diabetes, aging and neurodegeneration. DJ1 was recently identified as a deglycase in eukaryotic cells acting in early glycation products in DNA, RNA, and proteins or degrading glycating agents. However, the deglycase DJ1 function was not yet put in the context molecular mechanism of PD neurodegeneration nor the main cell types affected were identified. Here, using iPSC midbrain organoids model, we show that loss of DJ1 function causes the accumulation of advanced glycation products (AGEs) concomitantly with the increase in alpha-synuclein phosphorylation. These phenotypes are independent of glycolysis levels or accumulation of toxic glycolytic intermediaries. Prevention of AGE formation by the dicarbonyl scavenger aminoguanidine treatment decreased alpha-synuclein phosphorylation. Proteomics experiments in purified midbrain astrocytes showed increase reactivity related proteins and pro-inflammatory cytokines. Our results show that astrocytes lacking DJ1 activity display impairment in lysosomal proteolysis causing accumulation of aggregated proteins, alpha-synuclein, and AGEs. In addition, mix-genetic astrocytic co-cultures are able to reverse the proteolysis deficits observed in DJ1 KO midbrain neurons. In conclusion, astrocyte inability to clear toxic damaged proteins is detrimental for neuronal function and contributes to neurodegeneration observed in PD. The pathological mechanisms described involving astrocytes in this early onset PD model highlight new therapeutic targets for PD.

Keywords: Parkinson's disease, Brain Organoids, PARK7

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OPTIMIZING INDUCED NEURON CULTURES GENERATED FROM AGED HUMAN FIBROBLASTS FOR ELECTROPHYSIOLOGY STUDIES

Mozo, Maria - *Biology, California State University, San Marcos, Oceanside, CA, USA*

Abstract: Aging results in a greater risk for neurodegenerative diseases, therefore it is crucial to have a cell culture model that reflects this biological decline. The current induced pluripotent stem cell (iPSC) model provides a powerful method for studying the genetics of neurological disease. However, there are limitations due to loss of epigenetic modifications acquired throughout one's life and therefore they are not suited for disease modeling of healthy aging. In comparison to the iPSC model, the induced neuron (iN) model, where dermal fibroblasts are directly converted into neurons, more accurately reflects aging as it preserves epigenetic modifications. Indeed, the iN model can generate a highly enriched population of glutamatergic neurons, yet preliminary functional studies of iNs suggest that this method generates electrically immature neuronal cultures with limited action potential firing ability. In order to optimize the iN model to study ion channel function during aging, we tested the hypothesis that iNs need to be co-cultured with other cell types in order to form functional networks and support electrogenic maturation. We tested whether co-culturing iNs derived from aged human subjects with human astrocytes and other neuronal culture preparations can improve synaptic transmission and action potential firing. Fluorescent markers were used to distinguish cell types and monitor the relative ratios in co-culture conditions. We expect that co-culture conditions will result in a more electrically mature phenotype of neurons, displaying increased action potentials and increased excitatory and inhibitory postsynaptic events, thereby allowing us to further study the ion channel complement and functional changes associated with aging. This optimization of the current iN culture protocol will allow further insight into the study of functional changes due to aging.

Keywords: iPSC, neurodegenerative, induced neuron

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NEURO REGENERATION EFFECT OF PREEMPTIVE MESENCHYMAL STEM CELL THERAPY AND IPSC DERIVED MOTOR NEURON PROGENITOR CELLS TRANSPLANTATION IN A CONTUSIVE SPINAL CORD INJURY RAT MODEL.

Kim, Jangwoon - *Catholic iPSC Research Center (CiRC), The Catholic University of Korea, Seoul*
Rim, Yeri Alice - *Catholic iPSC Research Center (CiRC), Catholic University, Seoul, Korea*
Kim, Juryun - *Catholic iPSC Research Center (CiRC), Catholic University, Seoul, Korea*
Mo, Hyunkyung - *Catholic iPSC Research Center (CiRC), Catholic University, Seoul, Korea*
Han, Heeju - *Catholic iPSC Research Center (CiRC), Catholic University, Seoul, Korea*
Ju, JiHyeon - *Internal Medicine, Catholic University, Seoul, Korea*

Abstract: Spinal cord injury (SCI) causes dysfunction of motor, sensory and autonomic nervous system by traumatic injury to the spinal cord. Stem cell therapy for SCI is a promising treatment strategy for neuro regeneration. However, to present experimental evidence for therapeutic efficacy for stem cell transplantation in SCI is still lacking. Prior to clinical application of stem cell transplantation for SCI, prerequisites critical issues have to be addressed including transplantation of timing, method, efficacy of stem cell source and safety of transplanted cells. The purpose of this study is to investigate the effective neuro regeneration effect through preemptive human mesenchymal stem cell (hMSCs) transplantation and induced pluripotent stem cells (iPSCs) derived motor neuron progenitor cells (iMNPs) transplantation in a spinal cord injury animal model. Multiple intravenous injections of hMSCs in acute SCI increased neuronal differentiation of transplanted cells and decreased trauma-induced reactive gliosis in the injured site. The engraftments of transplanted iMNPs

were co-localized with OLIG2, ChAT and SMI-32 at chronic SCI phase. We were confirmed that axonal regeneration was promoted through effective inhibition of trauma-induced reactive gliosis when preemptive transplantation of hMSCs and iMNPs in spinal cord injury animal model.

Keywords: Spinal Cord Injury, Mesenchymal Stem Cells, iPSCs derived Motor Neuron Progenitor

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MODELLING THE TRANSCRIPTIONAL ROLE OF NIPBL IN CORNELIA DE LANGE SYNDROME

Somsen, Hedda B. - *Department of Cell Biology, Erasmus University Medical Center, Rotterdam, Netherlands*
Eigenhuis, Kristel - *Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands*
van den Berg, Debbie - *Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands*

Abstract: The six-layered mammalian neocortex is generated from a pool of radial glial progenitor cells in an inside-out manner, with early born neurons forming deep layers and later born neurons migrating radially to form superficial layers. Layer-specific neuronal identity is conferred by subtype-specific gene expression and connection patterns. We recently identified a defect in cortical neuronal migration as a possible cause for neurological defects in Cornelia de Lange Syndrome (CdLS), a developmental disorder characterized by intellectual disability, seizures, facial abnormalities and upper limb defects. Mutations in the cohesin loading factor NIPBL account for the vast majority (~70%) of genetically defined CdLS cases, which are thought to be caused by dysregulated gene expression. Indeed, we showed that NIPBL is a functional interaction partner of the neural transcription factor Zfp609 and the Integrator complex in mouse brain development. We now extend our analysis of NIPBL function in brain development to two- and three-dimensional human neural model systems. We have generated NIPBL heterozygous loss-of-function induced pluripotent stem (iPS) cells and neural progenitor cells (NPCs) by CRISPR/Cas9 genome editing. Consistent with frequently observed microcephaly in CdLS patients, NIPBL+/- NPCs show defects in proliferation and commitment to neuronal differentiation. We have mapped NIPBL binding sites in NPCs and used these to define NIPBL target genes that could contribute to CdLS-associated neurodevelopmental abnormalities. We are currently validating these findings in brain organoids, which recapitulate the cellular composition, cytoarchitecture, and gene expression profiles of human fetal brain development. Immunohistochemistry (IHC) for progenitor-, proliferation- and neuronal markers, along with gene expression analysis and single-cell RNA sequencing are performed at different time points in the differentiation process.

Funding Source: FEBS Long Term Fellowship, Marie Skłodowska Curie Individual Fellowship (#799214) and EUR Fellowship to D.L.C.v.d.B.
Keywords: NIPBL, Cornelia de Lange Syndrome, Brain Organoids

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MODELING GLIOBLASTOMA USING GENETICALLY ENGINEERED HUMAN STEM CELL-DERIVED CEREBRAL ORGANOIDS

Ishahak, Matthew - *Department of Medicine, Washington University, Saint Louis, MO, USA*
Qu, Xuan - *Department of Neurological Surgery, Washington University, St. Louis, MO, USA*
DeSouza, Patrick - *Department of Neurological Surgery, Washington University, St. Louis, MO, USA*
Kim, Albert - *Department of Neurological Surgery, Washington University, St. Louis, MO, USA*
Millman, Jeffrey - *Department of Medicine, Washington University, St. Louis, MO, USA*

Abstract: Glioblastoma (GBM) is the most common primary malignant brain tumor in adults and has a median survival of less than two years. GBM recurrence and treatment resistance are driven, in part, by the presence of glioblastoma stem-like cells (GSCs), which have the capacity for self-renewal, differentiation, and tumor formation. While patient-derived 3D tumor spheroids can recreate the cellular heterogeneity of GBM, the intrinsic genetic and epigenetic heterogeneity of primary patient GSCs hinder investigation of tumorigenesis. To model human GSCs, we genetically engineered human embryonic stem cells (hESCs) using CRISPR gene editing to introduce mutations in the TERT promoter and canonical GBM-associated genes that affect signal transduction and tumor suppression pathways. To validate the ability of genetically engineered GSCs to form tumors, engineered hESCs were differentiated into neural progenitor cells and transplanted into mice. Tumors were harvested and assessed using single-cell RNA sequencing (scRNAseq). Characterization of gene expression modules, based on transcriptomic GBM signatures established by the Cancer Genome Atlas Network, indicated that tumors recapitulate the transcriptional characteristics of GBM. To further characterize the role of GBM-associated genes in neural development and tumorigenesis, cerebral organoids were generated from both wild type (WT) and engineered mutant hESCs. During the differentiation, abnormal clustering and folding was observed in mutant organoids. Neural identity was confirmed by real-time PCR during organoid development in both WT and mutant conditions. Notably, expression of pluripotency markers was maintained during early development of mutant organoids, suggesting a sustained stem cell-like state, despite expression of neural markers. We performed scRNAseq on 1-month old cerebral organoids to identify transcriptional signatures of early tumorigenesis. These results suggest that aspects of GBM can be recapitulated in vitro using genetically engineered stem cell-derived organoids, and further analysis aims to elucidate the mechanisms of malignant transformation.

Keywords: Cerebral Organoid, Glioblastoma, Cancer

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LARGE-SCALE DIFFERENTIATION AND CHARACTERIZATION OF IPSC-DERIVED MOTOR NEURONS FROM ALS AND CONTROL SUBJECTS REVEALS CELL COMPOSITION AND SEX AS PRIMARY DRIVERS OF VARIATION

Workman, Michael J. - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, West Hollywood, CA, USA*
Lim, Ryan - *Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA, USA*
Wu, Jie - *Department of Biological Chemistry, University of California, Irvine, CA, USA*
Frank, Aaron - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Ornelas, Loren - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Panther, Lindsey - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Galvez, Erick - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Perez, Daniel - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Meepe, Imara - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Lei, Susan - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Gomez, Emilda - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Baxi, Emily - *Brain Science Institute, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
Consortium, Answer Als - *Answer ALS, New Orleans, LA, USA*
Thompson, Terri - *On Point Scientific Inc., San Diego, CA, USA*
Rothstein, Jeff - *Brain Science Institute, Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
Sareen, Dhruv - *Cedars-Sinai Biomanufacturing Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA*
Thompson, Leslie - *Department of Neurobiology and Behavior, Department of Psychiatry and Human Behavior, University of California, Irvine, CA, USA*
Svendsen, Clive - *Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA*

Abstract: Using induced pluripotent stem cells (iPSCs) to understand mechanisms of neurological disease holds great promise, but there is a lack of well curated lines. Answer ALS (www.answerals.org) has now created 1000 iPSC lines from amyotrophic lateral sclerosis (ALS) and control participant blood samples and differentiated over 400 into motor neurons (MNs) as a resource for the ALS and stem cell research communities. Differentiation of iPSCs to MNs was performed using a 32-day protocol in batches of 12 participants. Cells were processed for cell marker expression using high content imaging and bulk RNAseq analysis. All cultures generated approximately 60% TUJ1+ neurons with about 30% ISL1+ MNs, some non-neuronal cells, and no GFAP+ astrocytes. Unsupervised principal component analysis showed no clear separation of ALS vs. control participants and very few differentially expressed genes were detected between groups. However, correlation of principal components (PCs) with clinical and experimental covariates revealed high correlations of various PCs with %S100B+ cells and the sex of the participant. Further associations between covariates and gene expression were determined using a linear mixed model to estimate the proportion of variation in each gene attributable to technical and biological variables included in the metadata. Using this approach, we identified subsets of genes expressed in day 32 MNs that were highly correlated with our staining data and other experimental and clinical covariates, such as iPS cell of origin (T-cell vs. non T-cell) or C9orf72 expansion. This is the largest set of iPSC lines to be differentiated into motor neurons and suggests that cell composition and sex are important sources of variability that need to be carefully controlled for. The results of this study also shed light on other underappreciated technical and biological variables inherent in differentiating iPSCs into motor neurons at scale. Combined with the epigenomics, proteomics, and whole genome sequencing beginning to be publicly available for the same samples and participants analyzed in this study, these results should help further analysis of the Answer ALS dataset (dataportal.answerals.org) and provide novel insights into genes and pathways potentially affected in ALS.

Funding Source: Support for the Answer ALS project has been generously and kindly provided by too many sources to name here. Please see www.answerals.org/partners for the full list.
Keywords: Amyotrophic Lateral Sclerosis (ALS), neurodegenerative disease, motor neurons

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INTRANASAL DELIVERY OF INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL NSC SECRETOME IMPROVE FUNCTIONAL EXCITATORY SYNAPSES AND MEMORY ABNORMALITIES.

Mo, Hyunkyung - *College of Medicine The Catholic University of Korea, The Catholic University of Korea, Seoul, Korea*
Ju, Jihyeon - *Internalmedicime, The Catholic University of Korea, Seoul, Korea*

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder in the elderly, resulting in gradually destroyed cognitive abilities. The main pathological markers of AD are the Aβ plaques and the formation of Neurofibrillary tangles. Cell therapy has emerged as an alternative treatment of AD. Among many cell therapy studies, mesenchymal stem cells are being studied as the most popular candidate. However, studies on non-invasive treatments derived from induced pluripotent stem cells have not been conducted much. Here, we confirmed restoring memory and reducing amyloid-beta plaque accumulation of 5XFAD mice injected into the intranasal delivery ipsc derived cortical neural stem cell secretome(CNSC-SE) (5μg/g) once a week for 4 times. Interestingly, the cortical neuron treated CNSC-SE increases neuronal proliferation and dendritic structure and acquires electrical network activity and action potential bursts. The present study suggests that CNSC-SE is effective in reducing amyloid infiltration and restoring memory in early Alzheimer's disease.

Keywords: Induced Pluripotent Stem Cell-derived Cortical Neural stem cell, Alzheimer disease, Secretome

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ESTABLISHMENT OF A BLOOD-BRAIN BARRIER MODEL USING IPSC-DERIVED HUMAN CELLS

Carlson, Coby B. - *Discovery Research, FUJIFILM Cellular Dynamics, Madison, WI, USA*
Fiene, Rebecca - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Goedland, Madelyn - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Livingston, Megan - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Munn, Christie - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Xu, Xuan - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Tomotoshi, Kimihiko - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*
Vaidyanathan, Ravi - *Discovery Research, FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA*

Abstract: The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. For many years, the scientific community has needed a robust and human-relevant BBB model to evaluate barrier function and drug permeability in vitro, as well as to study neurodegenerative diseases that affect it. The power of iPSC technology provides access to the specialized cell types of the brain required to assemble such a model system, but the field has been challenged with generating cells that contain the appropriate markers, manufacturing a consistent supply of cells at-scale, and cryopreserving the material for subsequent on-demand use. As a leader in iPSC technology and innovation, FUJIFILM Cellular Dynamics, Inc. will present data on the generation, characterization, and utilization of 3 unique human iPSC-derived cell types for use in BBB model development, including astrocytes, brain microvascular endothelial cells (BMEC), and pericytes. Perhaps most notably, the differentiation and cryopreservation of BMEC to yield a cell type with distinctive morphological (cobblestone and tightly packed), structural (proper organization of tight junctions and appropriate expression of transporters), and functional (effective barrier formation) features that differ from other vascular endothelial cells lining peripheral blood cells has made the highest impact. Additionally, the optimization of media and supplements to enable long-term survival of all 3 cell types in co-culture and to promote superior functional performance in transendothelial electrical resistance (TEER) assays is a key factor in the establishment of a reliable BBB model. The potential to integrate this system with emerging organ-on-a-chip technologies and other 3D cell culture systems offers an exciting new capability for the drug discovery community to advance the understanding of BBB function with respect to human health and disease.

Funding Source: N/A
Keywords: Blood-Brain Barrier, Brain Microvascular Endothelial Cells, Pericytes

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IDENTIFYING REVIVAL STEM CELLS IN HUMAN EMBRYONIC STEM CELL-DERIVED CEREBRAL ORGANOIDS

Wang, Lu - *Department of Medical Biophysics, Donnelly Centre, University of Toronto, ON, Canada*
Heidman-Sivitilli, Adam - *Department of Biochemistry, Donnelly Centre, Univeristy of Toronto, ON, Canada*
Zhang, Hui - *Department of Biochemistry, Donnelly Centre, University of Toronto, ON, Canada*
Hernandez, Javier - *Department of Biochemistry, Donnelly Centre, University of Toronto, ON, Canada*
Attisano, Liliana - *Department of Biochemistry, Department of Medical Biophysics, Donnelly Centre, University of Toronto, ON, Canada*

Abstract: Neural stem cells (NSCs) are self-renewing, multipotent cells that initiate neurogenesis during both embryonic development and adulthood. During neurogenesis, NSCs give rise to progenitor cells known as radial glial (RG) cells, which will then expand and ultimately produce neurons and glial cells that form the central nervous system. Despite the well-studied concept of neurogenesis, the interaction of radial glial cell sub-populations and the exact origin of these RG cells remains poorly studied. Recently, a group of dormant stem cells in the intestine were discovered to replenish the intestinal stem cell pool upon injury. They are referred to as “revival stem cells (revSCs)”. Here, through single-cell sequencing, our group identified a subgroup of RG cells in human cerebral organoids (CO) that displayed a gene expression profile similar to the intestinal revSCs. Neurosphere assays were performed on revSC-like cells isolated via fluorescence-activated single cell sorting from dissociated human COs. The results revealed that these revSC-like cells displayed higher regenerative potentials as compared to remaining population of CO-derived cells. Their differentiation potentials and responses to induced injury will be explored further. By studying the properties of revSC-like cells, this work may reveal another level of hierarchy in neurogenesis that could be activated upon injury and used as a potential target of stem cell therapies for brain damages.

Funding Source: Medicine by Design - CFREF Program CIHR
Keywords: Neurogenesis, Cerebral Organoids, Regeneration

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GENERATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS FROM HUMAN ESCS AS A REGENERATIVE THERAPY FOR DEMENTIA

Salvador, Alison - *Experimental Medical Science, Lund University, Lund, Sweden*
Kirkeby, Agnete - *Experimental Medical Science, Lund University and Copenhagen University, Lund, Sweden*
Rifes, Pedro - *Neuroscience, Copenhagen University, Copenhagen, Denmark*
Zhang, Yu - *Cell Therapy Development and Manufacturing, Takara Bio Inc., Goteborg, Sweden*
Schörling, Alrik - *Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Alzheimer’s disease, dementia with Lewy bodies and Parkinson’s disease with dementia are common neurodegenerative diseases, mainly caused by the presence of intracellular neurotoxic aggregates. In the basal forebrain, this leads to de-

generation of acetylcholine-releasing neurons and cholinergic denervation, and thereby the typical disabling symptoms of dementias. Current available treatments merely provide temporary symptomatic relief. In this context, cell therapy is a promising approach to replace the lost basal forebrain cholinergic neurons (BFCNs) as a curative treatment option. In this study, we optimized the patterning of human pluripotent stem cells towards authentic BFCN progenitor cells, suppressing the differentiation of non-BFCN neuronal populations with similar developmental origin, such as the GABAergic interneurons. We validated the terminal maturation to a bona fide cholinergic neuronal fate by transplanting BFCN progenitors to the rat hippocampus and cortex. We also generate a rat model for Lewy bodies dementia by injecting in the basal forebrain a combination of preformed fibrils and an adenovirus carrying alpha synuclein. In summary, we optimized the patterning of BFCNs progenitor cells, which upon transplantation leads to mature grafted cells and integration in the structure of the hippocampus.

Funding Source: NSC-Reconstruct Horizon 2020 European Union Founding forResearch & Innovation
Keywords: Cholinergic neurons, Cell replacement therapy, Lewy bodies dementia

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DEVELOPING A NOVEL TREATMENT FOR SCHINZEL-GIEDION SYNDROME

Antonyan, Lilit - *Department of Human Genetics, McGill University and Douglas Mental Health University Institute, Montreal, PQ, Canada*
Peng, Huashan - *Research Centre, Douglas Mental Health University Institute, Montreal, QC, Canada*
Zhang, Xin - *Research Centre, Douglas Mental Health University Institute, Montreal, QC, Canada*
Zhang, Ying - *Research Centre, Douglas Mental Health University Institute, Montreal, QC, Canada*
Wu, Hanrong - *Research Centre, Douglas Mental Health University Institute, Montreal, QC, Canada*
Ernst, Carl - *Department of Human Genetics, McGill University and Douglas Mental Health University Institute, Montreal, QC, Canada*

Abstract: Missense mutations in a 4-aminoacid hotspot found in SETBP1 (SET binding protein 1) cause Schinzel-Giedion syndrome (SGS), an autosomal dominant gain-of-function (GOF) disease characterized by severe mental retardation, bone and renal abnormalities and neurological degeneration. There is currently no treatment for SGS and symptoms are so severe that children with SGS usually die within the first decade of life. To uncover the mechanisms underlying SGS, we made forebrain neural progenitor cells (NPCs) from 4 patients with SETBP1 GOF and their sex-matched controls through the use of induced pluripotent stem cells. We demonstrate that the mechanism of disease stems from the lack of degradation of SETBP1, leading to its accumulation in the cell. We found that the amount of SETBP1 present in GOF NPCs affects PP2A activity and critical downstream targets. At the cellular level, we found that GOF NPCs overproliferate and when differentiated into neurons, a proportion of the cells remains at the immature NPC state. Finally, we demonstrate that AntiSense Oligonucleotides (ASO) are effective at reducing expression of SETBP1 in GOF NPCs when treated at different doses and we propose them as a treatment for Schinzel-Giedion syndrome. These data are among the first models of SETBP1 neurodevelopmental syndromes and provide realistic therapeutic avenues for their treatment.

Funding Source: - CONACYT - Government of Mexico - FRQ - Fonds de Recherche du Québec - European Joint Programme on Rare Diseases
Keywords: PP2A signalling, Treatment, Rare disease

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CHARACTERISTICS OF HUMAN IPSC-DERIVED SENSORY NEURONS AND THEIR POTENTIAL AS AN ALTERNATIVE MODEL FOR DORSAL ROOT GANGLIA

Annand, Robert R. - *Marketing, REPROCELL, Beltsville, MD, USA*
Hiranuma, Minami - *Stem Cells, REPROCELL, Yokohama, Japan*
Okuda, Yuichi - *Stem Cells, REPROCELL, Yokohama, Japan*
Watanabe, Tomohisa - *Stem Cells, REPROCELL, Yokohama, Japan*

Abstract: Recently, the need for hiPSC-derived neurons as a research tool is increasing. To meet this need, we have developed hiPSC-derived sensory neurons. Sensory neurons are afferent nerves that transmit stimuli generated in sensory systems to the central nervous system as electrical signals. Primary afferent neurons induced by non-noxious and noxious stimuli are present in the dorsal root ganglia (DRG), and the DRG sensory neurons are used as in vitro model of nociceptive response. However, the often-used DRG derived from mouse or rat has the gives a low yield of neurons, and they are difficult to culture. We have induced hiPSC to differentiate to sensory neurons. This approach could solve the problems of interspecies differences and supply stability. We investigated expressions and drug responses by Multi-Electrode Array (MEA) to analyze the properties and functions of our sensory neurons. We further compared those parameters between hiPSC-derived sensory neurons and primary DRG. We have measured the expression level of sensory nerve-related genes by qPCR, and found that Peripherin, Brn3a, TRP channel and high-affinity neurotrophin receptor were significantly higher than hiPSC. It showed that our sensory neurons express all three well-known subtypes of high-affinity neurotrophin receptors: TRKA (nociceptor), TRKB (mechanoreceptor), and TRKC (proprioceptors). Furthermore, the expression level of Brn3a, TRPV1, TRPM8, and TRKB in hiPSC-derived sensory neurons were similar levels to those found in human DRG. In an electro-physiological analysis using MEA, exposing pain-causing drugs to neurons confirmed the drug response towards capsaicin and pain-related bradykinin in hiPSC-derived sensory neurons. The mean firing rate of our sensory neurons increased in a temperature-dependent manner. These results suggest that our hiPSC-derived sensory neurons could be an alternative model for primary hDRG.

Funding Source: This research was funded by REPROCELL, Yokohama, Japan.
Keywords: Sensory Neurons, in Vitro Model, Multi-Electrode Array

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AN ORGANOID MODEL TO INTERROGATE THE PATTERNING ROLE OF THE CHOROID PLEXUS IN HUMAN CEREBRAL CORTEX DEVELOPMENT

Hoffman, Ryan N. - *Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Felton, CA, USA*
Gaddis, Darhien - *Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA, USA*
Teodorescu, Mircea - *Electrical and Computer Engineering, University of California, Santa Cruz, CA, USA*
Haussler, David - *Genomics Institute, University of California, Santa Cruz, CA, USA*
Salama, Sofie - *Genomics Institute, University of California, Santa Cruz, CA, USA*

Abstract: The cerebral cortex (CC) is a highly organized laminar tissue that originates from a distinct region of multipotent neural stem cells of the neural tube. Although the development of the CC has been widely studied, the molecular mechanisms responsible for orchestrating the differentiation of progenitors into the diverse and spatially organized cell types of the CC has yet to be elucidated. The use of stem cell derived cerebral organoids (COs) as an in vitro model has emerged as a powerful tool for investigating human specific events of corticogenesis. During growth, CO tissue self-organizes neural rosettes that recapitulate the cellular identities and cytoarchitectural features of the CC yet fail to fully mature into molecularly distinct layer and region-specific neurons. Current CO protocols use exogenous patterning factors supplemented in the media to generate COs that successively develop isolated CC tissue that lack the interaction with neighboring tissues that occurs in vivo; most notably, interactions with the underlying ventricular system. I hypothesize that the interaction between the developing CC and choroid plexus (ChP) secreted molecules in the cerebrospinal fluid (CSF) plays an integral role in the patterning and maturation of cerebral cell types. To investigate this, a CO model with an accessible interior cavity that models appropriate apical-basal orientation must first be established. We are developing an aggregation technique that utilizes a biodegradable poly(lactic-co-glycolic acid) (PLGA) bead to engineer an interior cavity within COs and a patterning protocol that provides an exterior basal lamina-like coating to establish apical-basal polarization. PLGA beads are saturated with specific factors and coated in a hydrogel to deliver a sustained release of these factors over the first several weeks of patterning, providing molecular access to the apical surface of CC tissue during a crucial patterning period. Factors identified from transcriptomic data of the secretory mesenchymal cells of the ChP will be introduced to the interior cavity of COs and evaluated for their effect on the molecular composition of CO cell types, maturation trajectories of progenitor populations, and cytoarchitectural organization via single nuclei RNA sequencing and fluorescence immunohistochemistry.

Funding Source: CIRM EDUC4-12759 (RNH), NIH/NIMH R01MH120295 (SRS), Schmidt Futures Foundation SF 857 (D.H.), NSF 2034037 (M.T.), HHMI.
Keywords: Organoid, Choroid Plexus, Cerebrospinal Fluid

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AGE-RELATED MITOPHAGY IMPAIRMENT IN INDUCED NEURONS DERIVED FROM IDIOPATHIC PARKINSON'S DISEASE PATIENTS

Legault, Emilie - *Faculty of Pharmacy, Université de Montréal, QC, Canada*
Barker, Roger - *Department of Clinical Neurosciences, John van Geest Centre for Brain Repair & Department of Neurology, University of Cambridge, UK*
Drouin-Ouellet, Janelle - *Faculty of Pharmacy, Université de Montréal, QC, Canada*
Parmar, Malin - *Department of Experimental Medical Science, Wallenberg Neuroscience Center, Division of Neurobiology and Lund Stem Cell Center, BMC A11, S-221 84, Lund University, Lund, Sweden*

Abstract: Direct neural reprogramming allows the conversion of dermal fibroblasts to neurons, without passing by the pluripotency stage. This cell reprogramming maintains the age signature in the cell, making it an advantageous system in which to study age-related neurodegenerative disorders. Parkinson's Disease (PD) is characterized by the death of dopaminergic neurons and the accumulation of misfolded alpha-synuclein. The main risk factor for idiopathic cases is age. Therefore, we sought to use direct neural reprogramming as a new patient-specific model to study the PD pathophenotypes linked to aging. Mitophagy impairment and mitochondrial dysfunction contribute to the death of dopaminergic neurons observed in genetic forms of PD and are suspected to also contribute to idiopathic cases, although models exhibiting strong pathophenotypes for this type of PD are currently lacking. Here, we investigate the effect of mitophagy induction in induced dopaminergic neurons (iDANs) directly reprogrammed from dermal fibroblasts of idiopathic PD patients and healthy (H) donors. Our results show an accumulation of damaged mitochondria and of autophagic structures containing mitochondria specifically in the neurites of PD iNs following induction of mitochondrial damage, suggesting mitophagy impairment. Interestingly, the severity of this defect correlates with the age of the patients indicating the importance of maintaining the aging phenotype to further investigate mitochondrial dysfunction in iPD. Taken together, these results support the relevance of using direct neuronal reprogramming as a model to study donor-specific and age-related pathophenotypes in the context of neurodegenerative diseases. Because direct neuronal reprogramming can be easily used on large cohorts, it offers a new system in which the different molecular signatures of idiopathic PD can be studied.

Keywords: Direct neuronal reprogramming, Parkinson's disease, Mitophagy

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A PRACTICAL INDUCTION AND EVALUATION METHOD OF HUMAN SPINAL MOTOR NEURONS TO ACCELERATE DRUG SCREENING FOR ALS

Setsu, Selena - *Graduate School of Frontier Science, The University of Tokyo, Taito-ku, Japan*
Morimoto, Satoru - *Department of Physiology, Keio University School of Medicine, Tokyo, Japan*
Nakamura, Shiho - *Department of Physiology, Keio University School of Medicine, Tokyo, Japan*
Ozawa, Fumiko - *Department of Physiology, Keio University School of Medicine, Tokyo, Japan*

Takahashi, Shinichi - *Department of Neurology and Stroke, Saitama Medical University International Medical Center, Saitama, Japan*
Okano, Hideyuki - *Department of Physiology, Keio University School of Medicine, Tokyo, Japan*

Abstract: There have been several iPS cell-based disease modeling studies of neurodegenerative diseases (NDs) that used cells with obvious genetic abnormalities. The results of these studies are very important for clarifying the pathogenesis of NDs and developing treatment agents. However, it is important to note that most cases with NDs are sporadic patients without any apparent genetic defect. Disease progression and background of sporadic patients are highly heterogeneous and the iPS cell-based study of sporadic cases requires a large number of subjects to be analyzed. For drug screening an efficient and simple method for neural induction is needed to take advantage of the large collection of iPS cells derived from sporadic patients. We focused on amyotrophic lateral sclerosis (ALS) and developed a rapid and efficient method to induce human motor neurons of the spinal cord, the main locus of ALS pathology, from iPS cells. Our protocol takes only 2 weeks and the induction efficiency is more than 80%, whereas most previous protocols require more than a month for induction and the efficiency is typically less than 60%. In addition, we have established a highly accurate pathological evaluation system for drug screening by using time-lapse microscopy and machine learning to analyze the morphology including neurites and the viability of iPS cell derived neurons at single-cell resolution, while ensuring sufficiently large throughput. As cellular heterogeneity is another problem to be overcome in disease modelling with iPS cells, single-cell level analysis is a strong advantage. This platform should lead to further acceleration of iPS cell drug discovery for ALS.

Funding Source: This work was supported by MEXT Grant-in-Aid, Japan (grant no. JP20H00485 and JP21H05273) and AMED, Japan (grant no. 19bm0804003, 20bm0804003 and 21bm0804003, 20ek0109492, 21ek0109492 and 22ek0109492) to H.O.
Keywords: motor neuron, ALS, drug screening

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A FULLY PATTERNED HUMAN NEURAL TUBE MODEL USING MICROFLUIDICS

Fu, Jianping - *Mechanical Engineering, Biomedical Engineering, Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA*
Yan, Robin - *Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA*

Abstract: The human nervous system is the most complex but highly organized organ. The foundation of the complexity and organization of the human nervous system is laid down during regional patterning of the neural tube (NT), the embryonic precursor to the nervous system. Patterning of the NT results in distinct classes of neuronal progenitors specified in the NT along both the rostral-caudal (R-C) and dorsal-ventral (D-V) axes. Historically, studies of NT patterning rely on animal models. However, animal models do not reveal human-specific aspects of neurodevelopment. Recently, human pluripotent stem cell (hPSC)-based models of neurodevelopment are emerging as promising tools to study human neurodevelopment. However, existing models fail to recapitulate neural patterning along two orthogonal axes in a three-dimensional (3D) tubular geometry, a hallmark of NT development. Furthermore, they only model certain aspects of the development of either the human brain or the spinal cord (SC), but not both. Herein we report a hPSC-based, microfluidic NT-like structure (or μNTLS), whose development recapitulates critical aspects of neural patterning in the entire NT, including both the brain and SC regions, along both the R-C and D-V axes. Specifically, μNTLS recapitulates important early human NT development landmarks, including a single continuous central lumen enclosed by neuronal progenitor cells, patterned expression of canonical R-C and D-V regional markers including HOX genes, and emergence of the isthmic organizer (IsO) and neuromesodermal progenitors (NMPs). Transcriptomic analysis using scRNA-seq shows that the μNTLS contains various NT subdomains, including forebrain, midbrain, IsO, hindbrain, SC, NMP, neural crest (NC), roof plate, floor plate, and neurons. As a completely patterned NT model, our model offers a perfect experimental tool to study the lineage development dynamics of human NC cells with different R-C identities. Indeed, our data show that depending on their R-C identities, NC cells in μNTLS bifurcate into mesenchymal or Schwann cell fates, mimicking key aspects of NC developmental dynamics in vivo. Together, μNTLS offers a 3D tubular tissue architecture with an in vivo-like spatiotemporal cell differentiation and organization, helpful in studying human neurodevelopment and diseases.

Funding Source: This work is supported by the Michigan-Cambridge Collaboration Initiative, the University of Michigan Mcubed Fund, the 21st Century Jobs Trust Fund, the National Science Foundation, and the National Institutes of Health.
Keywords: Human pluripotent stem cells, Neural tube patterning, Microfluidics

TOPIC: PANCREAS

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HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED B CELLS IN THE EFFECTIVE THERAPEUTIC TREATMENT OF TYPE 1 DIABETES IN A MOUSE MODEL

Ottewell, Julia - *Biology, California State University, San Marcos, Oceanside, CA, USA*

Abstract: Type 1 diabetes mellitus (T1D) is a chronic, autoimmune disease that affects over 371 million people worldwide and leads to immense socio-economic and societal burdens. The use of human induced pluripotent stem cell (hiPSC) therapy for the replacement of dysfunctional β cells of the pancreas provides significant therapeutic efficacy. Yet this goal remains limited due to the difficulty of producing autologous and uniformly differentiated, insulin-expressing lines. Here, we follow on previous work (2016 Ni Y et al, Curr Protoc Stem Cell Biol) and report efficient mRNA-based reprogramming and production of laboratory and medium scale numbers of cGMP-grade human b cells with nearly homogenous identity. We furthermore assessed in vitro characteristics and determined the cells response in glucose-stimulated insulin secretion (GSIS) assays. Next, using a streptozotocin (STZ)-induced and immunosuppressed T1D mouse model, human b cells were injected into the hepatic portal vein in cohorts of mice, and the animals were followed longitudinally over several weeks at a time. STZ T1D mice showed consistently high elevation (400-600 mg/dl) of glucose within 24-48 hours days of STZ delivery without b cells. High glucose levels were decreased to baseline (80-150 mg/dl) and maintained upon administration of insulin. Most importantly, b cell-injected animals also showed normalization of

non-fasting glucose levels to normal or prediabetic (150-250 mg/dl) for several weeks without the need for exogenous insulin administration. Histological examination revealed the presence and localization of intact and surviving b cells that were positive for b cell and human cell markers, insulin, and other metrics. Overall, these early findings support future investigation into optimizing personalized iPSC-derived therapies for T1D diabetes.

Keywords: Type 1 diabetes mellitus, induced pluripotent stem cell, beta cells

TOPIC: PLURIPOTENT STEM CELLS

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THE ISOGENIC KIDNEY GLOMERULUS CHIP ENGINEERED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Roye, Yasmin - *Biomedical Engineering, Duke University, Durham, NC, USA*
Bhattacharya, Rohan - *Biomedical Engineering, Duke University, Durham, NC, USA*
Mou, Xingrui - *Biomedical Engineering, Duke University, Durham, NC, USA*
Zhou, Yuhao - *Biomedical Engineering, Duke University, Durham, NC, USA*
Burt, Morgan - *Biomedical Engineering, Duke University, Durham, NC, USA*

Abstract: The number of people afflicted with chronic kidney disease in the U.S. continues to increase, meanwhile, development of targeted therapeutics to treat this disease has been limited. To better understand disease mechanisms and unveil novel therapeutic targets, in vitro models of the kidney have been engineered including the Organ Chip (or organ-on-a-chip). Organ chips that aimed to model the kidney's glomerulus were typically reconstituted with cells derived from animals, or from humans of heterogenous sources. These approaches were disadvantageous because animal models fail to recapitulate human physiological responses and heterogeneous mixtures of human cells do not accurately reflect the genetic profile and/or functional characteristics of any one patient donor. Here, we present a novel glomerulus chip that is derived from human induced pluripotent stem (iPS) cells of a single patient. To engineer this model, isogenic iPS cells were first bifurcated into two distinct lineages which resulted in the generation of vascular endothelium and glomerular epithelium (podocytes). Within the microfluidic chip system, both endothelial- and epithelial cell layers successfully mimicked the structure and some essential functions of the glomerular filtration barrier. The endothelium and podocytes were positive for lineage-specific markers, PECAM-1/VE-Cadherin and Nephrin/Podocin, respectively. Additionally, both cell layers deposited the most abundant basement membrane protein, collagen (IV). Together, the endothelium-basement membrane-epithelium barrier within the chip selectively filtered small (Inulin) and large (Albumin) molecules. The barrier's permselectivity was disrupted following administration of a clinically relevant dose of a nephrotoxic, chemotherapeutic drug, resulting in significant albuminuria. Overall, the isogenic glomerulus chip takes advantage of human iPS cells' unlimited self-renewal, differentiation into almost any cell type, and their preservation of the molecular and genetic profile of the donor. The isogenic glomerulus chip has the potential to advance kidney precision medicine and serve as a blueprint to establish other patient-specific organ-on-a-chip and 'body-on-a-chip' models.

Keywords: Organ-on-a-chip, Glomerulus, Isogenic

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MODELING THROMBOCYTOPENIA ABSENT RADIUS SYNDROME DURING DEVELOPMENT USING INDUCED PLURIPOTENT STEM CELLS

Nations, Catriana C. - *Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA, USA*
Pavani, Giulia - *Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
French, Deborah - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
Gadue, Paul - *Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, USA*

Abstract: Thrombocytopenia absent radius (TAR) syndrome is a rare congenital disorder resulting in skeletal defects, severely reduced numbers of mature megakaryocytes (MKs), and thrombocytopenia. TAR is caused by compound heterozygous mutations in the ubiquitously expressed gene RBM8A, leading to reduced expression of its encoded protein, Y14. Y14, a core member of the exon-junction complex, is important for post-transcriptional mRNA regulation and splicing, but it has no known roles in MK biology. Previous studies have identified a role for Y14 in apoptosis and cell cycle regulation, but it is unclear whether this is the mechanism responsible for the MK phenotype in TAR patients. In vivo, MKs regulate the isoform expression of specific apoptotic factors and diverge from canonical cell cycle progression during polyploidization as they mature and produce platelets. However, it is unknown if MKs regulate these pathways in a lineage-specific or developmental stage-specific manner. Therefore, we hypothesize that Y14 depletion in TAR syndrome impairs the maturation of MKs through altered cell cycle and apoptosis regulation. To test this hypothesis, we have generated two induced pluripotent stem cell (iPSC) lines from unrelated TAR patients. Due to the complexity of the genetic defect, isogenic corrected lines were created by targeting the AAVS1 locus with a CD43-promoter-driven RBM8A construct that is expressed at the hematopoietic progenitor cell (HPC) stage of differentiation. We can successfully generate MKs from these lines that can be assessed for impaired maturation, function, cell cycle progression, and apoptosis. Using these MKs, we show a 3-fold decrease in MK number and 2-fold decrease in proplatelet formation from the patient line compared to its isogenic corrected line and an unrelated control. Ongoing studies are investigating the gene expression and alternative splicing changes as well as potential mechanisms for these phenotypes. These tools provide the means to dissect the MK-specific mechanism of TAR syndrome, leading to a better understanding of this lineage-specific phenotype and MK maturation during development.

Funding Source: This study was supported in part by T32-HD083185.
Keywords: induced pluripotent stem cells, disease model, megakaryopoiesis

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MATERNAL USE OF VAPING LIQUIDS INDUCE DETRIMENTAL SECRETOME ALTERATION AND METABOLIC REPROGRAMMING IN FETAL VASCULATURE LEADING TO ITS DESTABILIZATION AND APOPTOSIS

Ho, Mirabelle - *Stem Cells, CReATe Fertility Centre, Toronto, ON, Canada*
Ho, Miriel - *Stem Cells, CReATe Fertility Centre, Toronto, ON, Canada*
Librach, Clifford - *Fertility, CReATe Fertility Centre, Toronto, ON, Canada*

Abstract: The misconception of vaping as a healthier alternative to cigarettes has led to its increased use during pregnancy. However, the effect of vaping liquid (VL) within maternal circulation on fetal vasculature remains unknown. Patent vasculature requires fragile endothelial cell (EC) neovessels being stabilized by smooth muscle cells (SMCs). We posit that VL exposure will destabilize EC networks and promote apoptosis via disruption of reciprocal regulation with SMCs. Immature iECs (>95%, CD144, CD31) and iSMCs (>90%, a-SMA, Calponin) differentiated from hiPSCs allows the modelling of VL effect on fetal vasculature. To simulate acute exposure, iECs were treated twice daily over 10 days with Mint or Peanut-Caramel VL, representing Simple (S-VL) and Complex (C-VL) flavorings, respectively. Both S-VL and C-VL are nicotine-free. Immunostaining observed formation of G3BP1+ stress granules and disruption of the actin cytoskeleton following exposure to S-VL (>2-fold) and C-VL (>4-fold). S-VL and C-VL treatment also increased apoptosis, and markedly reduced mitochondria membrane potential (ΔΨM) of iECs, as assessed by Annexin/PI and JC-10 flow cytometry, respectively. CellRox assay also noted an increase in iECs' reactive oxygen species (ROS) production (S-VL vs. C-VL: 55% vs. 73%, $p< 0.05$). Increase in cleaved Caspase-3-and-9 protein expression associated with severe functional impairment of S-VL and C-VL treated iECs. Premature network collapse (< 12Hr) resulting from poor apposition of iECs with fresh iSMCs, and reduced migratory ability of iECs was observed in Matrigel and Scratch-wound assays. Intriguingly, iSMCs cultured in S-VL and C-VL treated iEC-derived conditioned media (i.e. iEC-CM) demonstrated decreased ΔΨM and increased ROS production. Functional impairment of iEC-CM-cultured iSMCs was also evidenced by markedly inferior network formation following co-culture with fresh ECs on Matrigel. Thus, independent of nicotine, VL flavorings are able to adversely alter iEC's metabolic profile, contributing to reduction in angiogenic ability and viability. Importantly, ability of VL-treated secretome to mediate metabolic reprogramming and subsequent impairment of iSMC's network-stabilization ability, potentially represents a novel mechanism by which VL induces vascular destabilization.

Keywords: Vasculature, Vaping, hiPSC

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HUMAN IPSC-DERIVED NEURONS AND BRAIN ORGANOIDS FROM CJD INDIVIDUALS WITH THE E200K MUTATION RECAPITULATE PATHOLOGICAL HALLMARKS OF THE DISEASE

Gojanovich, Aldana D. - *Center for Regenerative Medicine, Boston University, Boston, MA, USA*
Le, Nhat - *Cancer Biology and Genetics, The Ohio State University, Columbus, OH, USA*
Mercer, Robert - *Biochemistry, Boston University, Boston, MA, USA*
Anane, Alice - *Israel CJD Foundation, Israel CJD Foundation, Haifa, Israel*
Harris, David - *Biochemistry, Boston University, Boston, MA, USA*
Mostoslavsky, Gustavo - *Center for Regenerative Medicine, Boston University, Boston, MA, USA*

Abstract: Genetic Creutzfeldt-Jakob disease (gCJD) caused by the E200K mutation on the PRNP gene encoding the prion protein (PrPc) is the most common subtype of genetic prion disease worldwide. We report the establishment of the largest CJD E200K-specific iPSC library and their differentiation towards cortical neurons and brain organoids to characterize the mutant PrP. Samples for reprogramming were obtained from 22 individuals of a large family including carriers and non-carriers of the E200K mutation. Some of these iPSC lines were selected for differentiation into pyramidal cortical neurons, a brain region highly affected by this mutation. A systematic comparison of hiPSC-derived neurons expressing E200K PrP to those expressing only the normal form revealed the presence of disease-relevant phenotypes. By analyzing the biochemical properties of PrP and Tau protein, a microtubule-associated protein involved in neurodegenerative diseases known as tauopathies, we found that the E200K hiPSC-derived neurons accumulate pathological forms of PrP which co-localize with paired helical filaments of tau protein. Neurofibrillary tangle-like aggregates of tau and neurofilament were also observed. At the postsynaptic site, N-Methyl-D-aspartic acid receptors and postsynaptic density 95 protein colocalization is disrupted in neurons expressing E200K PrP. Besides, through differentiation of these iPSC lines into brain organoids we were able to obtain a stable 3D platform for disease modeling. Characterization of these cultures revealed variations in organoids size of those with the E200K mutation compared with the non-mutants, being significantly smaller. Our study shows, for the first time, that hiPSC-derived neurons and brain organoids expressing endogenous levels of mutant PrP can model certain aspects of human prion disease, offering a powerful platform for investigating subtype pathologies and testing putative therapeutics.

Funding Source: R21NS111499-01
Keywords: CJD/Prions, hiPSC, Brain Organoids

406

GENOME-WIDE ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS IN HUMAN PLURIPOTENT STEM CELLS

Sarel-Gallily, Roni - *Genetics, The Hebrew University, Jerusalem, Israel*
Golan Lev, Tamar - *Genetics, The Hebrew University of Jerusalem, Israel*
Yilmaz, Atilgan - *Genetics, The Hebrew University of Jerusalem, Israel*

Sagi, Ido - *Genetics, The Hebrew University of Jerusalem, Israel*
Benvenisty, Nissim - *Genetics, The Hebrew University of Jerusalem, Israel*

Abstract: Haploinsufficiency describes a phenomenon where one functioning allele of a gene in a diploid cell or organism is insufficient for a normal phenotype. Although haploinsufficiency underlies several human diseases, the effect of haploinsufficiency on human embryonic stem cell (hESC) growth and proliferation has not been thoroughly studied. Here, we aimed to identify genes affecting the normal growth and proliferation of hESCs when one of their two alleles is lost. To establish a genome-wide loss-of-function screening for heterozygous mutations, we fused normal haploid hESCs with a library of mutant haploid hESCs. We have identified over 600 genes with a negative effect on hESC growth in a haploinsufficient manner and characterized them as genes that show less tolerance to mutations, more conservation during evolution and depletion from telomeric edges and X chromosome. Interestingly, a large fraction of these haploinsufficiency genes is associated with the extra-cellular matrix and the plasma membrane. We have revealed an enrichment of genes causing haploinsufficiency disorders within WNT and TGF-beta signal transduction pathways. We could thus identify haploinsufficiency-related genes and pathways that show growth retardation in early embryonic cells, suggesting dosage-dependent phenotypes in hESCs. We further investigated two disease-related genes with known haploinsufficiency effects and have discovered early growth and molecular effects of heterozygous mutations in those genes. Overall, we have constructed a novel model system for studying haploinsufficiency, discovered early effects of disease-related haploinsufficiency genes and identified important dosage-dependent genes and pathways involved in hESC growth and survival. This genome-wide heterozygous loss-of-function library allows us to further study haploinsufficiency phenotypes in additional fields, such as differentiation and drug resistance.

Keywords: Haploinsufficiency, Genetic Screening, Human Embryonic Stem Cells

408

GENERATION OF MOUSE-HUMAN CHIMERIC EMBRYOS USING NAIVE STATE HUMAN PLURIPOTENT STEM CELLS

Feng, Jian - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*
Zhang, Boyang - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*
Li, Hanqin - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*
Hu, Zhixing - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*
Jiang, Houbo - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*
Stablewski, Aimee - *Gene Targeting and Transgenic Resource, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA*
Marzullo, Brandon - *Genomics and Bioinformatics Core, State University of New York at Buffalo, NY, USA*
Yergeau, Donald - *Department of Physiology and Biophysics, State University of New York at Buffalo, NY, USA*

Abstract: Naïve human pluripotent stem cells (hPSCs) enable the generation of mature human cells of all three germ layers in mouse-human chimeric embryos. Here, we describe a method for generating mouse-human chimeric embryos by injecting naïve hPSCs converted from the primed state. Primed hPSCs are treated with an mTOR inhibitor (Torin1) for 3 hr and dissociated to single cells, which are plated on mouse embryonic fibroblasts in 2iLI medium, a condition essentially the same for culturing mouse embryonic stem cells (mESCs). After 3-4 days, bright, dome-shaped colonies with mESC morphology are passaged in 2iLI medium. Established naïve hPSCs are injected into mouse blastocysts, which produce E17.5 mouse embryos containing 0.1-4% human cells as quantified by next-generation sequencing of 18 S ribosomal DNA amplicons. The method is suitable for studying the development of hPSCs in mouse embryos and may facilitate the generation of human cells, tissues, and organs in animals. Furthermore, the naive hPSCs can be used to generate human blastoid-like structures in vitro in a defined medium. In the presence of an RNA splicing inhibitor, the naive hPSCs acquired the expression of many 2C genes, such as ZSCAN4. These results show the usefulness of naive hPSCs in modeling early human development in vitro.

Funding Source: The work was supported by NYSTEM contracts C028129 (J.F.), C029556 (J.F.), C30290GG (fellowship for H.L.), and Buffalo Blue Sky Initiative (J.F.).
Keywords: naive state, human pluripotent stem cells, interspecies chimeras

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CELL-DERIVED EXTRACELLULAR MATRICES RAPIDLY ELICIT MATURE PHENOTYPES FROM IPSC-DERIVED SOMATIC CELLS IN 2D CULTURE

Block, Travis J. - *Technology, StemBioSys, Inc., San Antonio, TX, USA*

Abstract: Human primary cells secrete extracellular matrices in vitro that retain properties of the tissue of origin. These tissue-specific ECMs promote maturation of iPSC-derived somatic cells in 2D culture. At StemBioSys we have developed processes for reproducibly manufacturing matrices capable of promoting rapid and spontaneous maturation of iPSC-derived cardiomyocytes, neurons, hepatocytes, and beta cells in high-throughput. 7 days after seeding on cardiomyocyte maturation matrix, iPSC-derived cardiomyocytes exhibit a mature phenotype, with rod-shaped morphology, 20-30% of cells being binucleated, and dramatically unregulated cTnI expression ($p<0.00001$). Glutamatergic neurons seeded onto an astrocyte-derived matrix rapidly cluster and begin forming synapses. After 4 days in culture, neurons on the astrocyte derived matrix have substantially more synapses relative to controls ($p<0.01$). Hepatocytes on a stellate cell-derived matrix exhibit decreased alpha-feto-protein expression ($p<0.01$) and increased urea secretion ($p=.08$). Moreover, iPSCs from patients with inherited diseases exhibit the disease phenotype in 2D culture. Thus, this technology has the potential to dramatically improve the utility of iPSCs in preclinical drug testing applications for efficacy or toxicity screening.

Funding Source: N/A
Keywords: extracellular matrix, disease modelling, cardiomyocyte

TOPIC: CARDIAC

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INVESTIGATING THE FUNCTIONAL COMMUNICATION BETWEEN SYMPATHETIC NEURONS AND HUMAN IPSC-DERIVED CARDIOMYOCYTES (HIPSC-CMS) IN VITRO

Mohammadi, Neda - *National Heart and Lung Institute, Imperial College London, UK*
Sanchez-Alonso, Jose - *National Heart and Lung Institute, Imperial College London, UK*
Harding, Sian - *National Heart and Lung Institute, Imperial College London, UK*
Gorelik, Julia - *National Heart and Lung Institute, Imperial College London, UK*

Abstract: Sympathetic neurons are essential for the control of cardiac inotropy, chronotropy and dromotropy. They release the chemical neurotransmitter norepinephrine (NE) through axon terminals across synapses, to activate β-Adrenoceptors on the surface of cardiomyocytes. Given the importance of this communication for normal cardiac function, a thorough in vitro understanding is required as a prerequisite for translational and therapeutic applications, focusing on sympathoadrenergic conditions. Here we investigate this communication through the coculture of sympathetic neurons with hiPSC-CMs. Our results indicate that neurons establish functional communication with hiPSC-CMs as early as 2 days in coculture. Surface topographical studies by Scanning Ion Conductance Microscopy showed no significant change in neuro-cardiac junction size up to 15 days (2-4 days volume: 17.75±2.88 μm3, 8-11 days volume: 13.92±2.04 μm3, and 13-15 days volume: 14.59±3.93 μm3, N=3). Following the establishment of synaptic communication, functional cell-cell communication was investigated between 7 and 10 days in co-culture using a novel high throughput device, CytoCypher. This was used to simultaneously record changes in contraction and calcium in the hiPSC-CMs incubated with the Fura-2, AM dye at baseline, and after the sequential application of 1μM and 10μM nicotine, whilst pacing using a field stimulator at 1 Hz throughout. In paced experiments, data showed a significant increase in the peak height (amplitude) of hiPSC-CM contractions in coculture ($p < 0.05^{***}$, N=3). Enhanced contractile activity in coculture was supported by a significant increase in departure and return velocity ($p < 0.05^{***}$, N=3). Calcium transient amplitude was also significantly higher in the presence of sympathetic neurons ($p < 0.05^{***}$, N=3). Spontaneously contracting cells showed a concentration-dependent increase in hiPSC-CM contraction frequency after nicotinic neuronal stimulation was observed, with contraction rate higher in cocultures at baseline ($p < 0.05^{**}$, N=6). Overall, our data suggests that the neuro-cardiac junction produces a functional effect in hiPSC-CMs resulting from sympathetic innervation, and an exciting new possibility for the development of hiPSC-CMs towards a more physiological condition observed in the human body.

Keywords: Cardiomyocyte, Stem Cell, Sympathetic Neuron

TOPIC: EARLY EMBRYO

926

MINI-SPINE EXPLANT CULTURE: AN EX-VIVO MOUSE MODEL SYSTEM FOR FUNCTIONAL STUDIES ON THE NOTOCHORD AND NUCLEUS PULPOSUS

Au, Tiffany Y.K. - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Wu, Ron - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*
Leung, Victor - *School of Clinical Medicine, University of Hong Kong, Hong Kong*
Cheah, Kathryn - *School of Biomedical Sciences, University of Hong Kong, Hong Kong*

Abstract: The nucleus pulposus (NP) in the core of the intervertebral disc (IVD) is critical for maintaining shock-absorbing function, protecting the spine from injury and degeneration. The NP originates from the embryonic notochord, that emerges soon after gastrulation and acts as the early axial skeleton. As the vertebrae form, the notochord undergoes remodelling, and segmentation to form the NP. Little is known of the molecular control/changes that occur during the transition from the notochord to NP. Here we developed two systems to provide insights into the transition : a double transgenic mouse model Foxa2mNE-Cre/Z/EG for lineage tracing of notochordal cells and an ex-vivo model system of notochord development in explant culture (Mini-spine). Using these systems we confirmed the contribution of notochordal cells to the mature NP. The ex-vivo model system recapitulated the segmentation of the notochord and its development into NP. Further, to understand the molecular dynamics of the transition, we studied the global transcriptomic changes that occur, as the notochord transitions to the early NP and matures down to single cell resolution. The knowledge gained from the transcriptomes aided a robust protocol for directed in vitro differentiation of mouse embryonic stem cells (mESC) to the notochordal lineage and provided new insights into the differentiation process. Furthermore using the Foxa2mNE-Cre/Z/EG model, we implicate the transformation of notochordal descendants to a fibroblastic state in injury induced disc degeneration.

Funding Source: RGF Collaborative Research Fund (CRF) 2019/2020 (C7044-19G)
Keywords: Spine Explant, Ex vivo model, differentiation and degeneration

TOPIC: EPITHELIAL_GUT

928

MODELING HOST-PARASITE INTERACTION IN CHAGAS DISEASE WITH MURINE INTESTINAL ORGANOIDS

Daghero, Hellen - *Cell Biology Unit, Institut Pasteur Montevideo, Uruguay*
Pagotto, Romina - *Cell Biology Unit, Institut Pasteur Montevideo, Uruguay*
Medeiros, Andrea - *Redox Biology of Trypanosomes, Institut Pasteur Montevideo, Uruguay*
Quiroga, Cristina - *Redox Biology of Trypanosomes, Institut Pasteur Montevideo, Uruguay*

Comini, Marcelo - *Redox Biology of Trypanosomes, Institut Pasteur Montevideo, Uruguay*
Bollati Fogolin, Mariela - *Cell Biology Unit, Institut Pasteur Montevideo, Montevideo, Uruguay*

Abstract: Chagas disease (CD) is a potentially life-threatening illness caused by the parasite Trypanosoma cruzi (T. cruzi). With around seven million people infected worldwide and over 10,000 deaths per year, CD is a major public health issue in Latin America. The main route of transmission to humans is through a triatomine bug (vector-borne) and, to a minor extent, by blood transfusion, organ transplantation, laboratory accidents, congenitally and orally (food-borne). The acute phase of CD presents mild symptoms. If left untreated, it develops into a long-lasting chronic illness, characterized by severely impaired cardiac, digestive, and neurological functions. The intestinal tissue appears to have a key role during oral transmission and chronic infection of CD. In these immune-privileged reservoirs, dormant/quiescent parasites have been suggested to contribute to disease persistence, infection relapse, and treatment failure. However, the interaction between the intestinal epithelium and T. cruzi has not been examined in depth, in part, due to the lack of in vitro models resembling the biological and structural complexity of this organ. Therefore, to understand the pathophysiological role played by the intestinal tissue during transmission and chronic infection, we evaluated the progression of T. cruzi infection of murine colon organoids. In order to model CD, 3D and 2D systems of murine intestinal organoids were infected with T. cruzi Dm28c, a strain that has been associated with high virulence and oral outbreaks. At different time points, the presence and load of parasites in the organoids, as well as the host cell morphology, were evaluated by confocal microscopy and compared to those obtained with a classical infection model (Vero cells). We show that the parasite invades and replicates in intestinal epithelial primary cells grown as intact organoids (3D) and monolayers (2D). The permissiveness to pathogen infection differed markedly between the primary and the tumoral (Vero) cells. So far, this represents the first evidence of the potential of these nearly physiological cellular systems to study host-pathogen interaction for CD and/or for the future evaluation of anti-chagasic drugs.

Funding Source: Resarch and Innovation National Agency (ANII): FMV_1_2019_1_156213 grant and PhD ANII Fellowship POS_NAC_2019_1_157518 (HD)
Keywords: Host-parasite interaction, Chagas disease, Intestinal organoids

TOPIC: EYE AND RETINA

930

NAD-RELATED METABOLITES ATTENUATE THE PHENOTYPE OF CORNEAL ENDOTHELIAL CELLS DERIVED FROM FUCHS CORNEAL ENDOTHELIAL DEGENERATION PATIENTS DERIVED-IPS CELLS

Inagaki, Emi - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Sayano, Tomoko - *Keio University School of Medicine, Department of Ophthalmology, Tokyo, Japan*
Sugai, Etsuko - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Fusaki, Noemi - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Yamazaki, Risa - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Rusch, Robert - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Hatou, Shin - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Negishi, Kazuno - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Tsubota, Kazuo - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*
Okano, Hideyuki - *Keio University School of Medicine, Department of Physiology, Tokyo, Japan*
Shimmura, Shigeto - *Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan*

Abstract: As we approach the era of 100 years of life, extending healthy life expectancy has become an important proposition for science in the context of SDGs initiatives. In recent years, there has been an urgent need to establish novel methods to control aging diseases through an anti-aging medicine approach that targets common factors in aging to prevent and treat aging-related diseases. The corneal endothelium is the transparent layer of the eye. The corneal endothelium is an important cell that contributes to the transparency of the eye, and since it does not divide after birth, a decrease in the number of cells can lead to bullous keratopathy, a condition similar to blindness. First, we established disease-specific induced pluripotent stem cells (iPSCs) from patients with Fuchs' corneal endothelial dystrophy (FECD), which is a representative disease that presents with bullous keratopathy. Secondly, the iPSCs were able to differentiate into corneal endothelial cells in both healthy subjects and the disease group successfully. In the FECD group, we observed phenotypes such as increased DNA damage, increased oxidative stress, and increased ER stress in the induced cells. Finally, when NAD-related metabolites were administered to the iPSCs corneal endothelial cells, the intracellular NAD concentration increased, and the intracellular reduced glutathione increased, indicating that various disease phenotypes such as DNA damage, oxidative stress, and ER stress were improved. In the future, we hope to apply NAD-related metabolites as a preventive agent for ocular aging by administering them intravitreally and modifying the NAD concentration in the eye to prevent disease phenotypes.

Funding Source: This work was supported by grants from Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS) to Shigeto Shimmura (19K09978), Japan Cornea Society (Novartis Pharma Grants 2020) to Emi Inagaki.
Keywords: Fuchs endothelial corneal dystrophy, iPSCs, NAD-related metabolites

TOPIC: IMMUNE SYSTEM

932

ANATOMICALLY DISTINCT FIBROBLAST SUBSETS DETERMINE SKIN AUTOIMMUNE PATTERNS

Chen, Daoming - *National Institute of Biological Sciences, Beijing, China*
Xu, Zijian - *National Institute of Biological Sciences, Beijing, China*
Hu, Yucheng - *Capital Normal University, Beijing, China*
Jiang, Kaiju - *National Institute of Biological Sciences, Beijing, China*
Huang, Huanwei - *National Institute of Biological Sciences,*

Beijing, China
Du, Yingxue - *National Institute of Biological Sciences, Beijing, China*
Wu, Wenbo - *National Institute of Biological Sciences, Beijing, China*
Wang, Jiawen - *National Institute of Biological Sciences, Beijing, China*
Sui, Jianhua - *National Institute of Biological Sciences, Beijing, China*
Wang, Wenhui - *Peking University Third Hospital, Beijing, China*
Zhang, Long - *Peking University Third Hospital, Beijing, China*
Li, Shuli - *Department of Dermatology, Xijing Hospital, Xi'an, China*
Li, Chunying - *Department of Dermatology, Xijing Hospital, Xi'an, China*
Yang, Yong - *Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, China*
Chang, Jianmin - *Department of Dermatology, Beijing Hospital, Beijing, China*
Chen, Ting - *National Institute of Biological Sciences, Beijing, China*

Abstract: The skin serves as a physical barrier and an immunological interface that protects the body from the external environment. Aberrant activation of immune cells can induce common skin autoimmune diseases such as vitiligo, which are often characterized by bilateral symmetric lesions in certain anatomic regions of the body. Understanding what orchestrates the activities of cutaneous immune cells at an organ level is necessary for the treatment of autoimmune diseases. Here we identify subsets of dermal fibroblasts that are responsible for driving patterned autoimmune activity, by using a robust mouse model of vitiligo that is based on the activation of endogenous auto-reactive CD8+ T cells that target epidermal melanocytes. Using a combination of single-cell analysis of skin samples from patients with vitiligo, cell-type-specific genetic knockouts and engraftment experiments, we find that among multiple interferon-γ (IFNγ)-responsive cell types in vitiligo-affected skin, dermal fibroblasts are uniquely required to recruit and activate CD8+ cytotoxic T cells through secreted chemokines. Anatomically distinct human dermal fibroblasts exhibit intrinsic differences in the expression of chemokines in response to IFNγ. In mouse models of vitiligo, regional IFNγ-resistant fibroblasts determine the autoimmune pattern of depigmentation in the skin. Our study identifies anatomically distinct fibroblasts with permissive or repressive IFNγ responses as the key determinant of body-level patterns of lesions in vitiligo, and highlights mesenchymal subpopulations as therapeutic targets for treating autoimmune diseases.

Keywords: Fibroblast, Vitiligo, Autoimmune pattern

TOPIC: LIVER

934

PHENOTYPICAL, FUNCTIONAL, AND TRANSCRIPTOMIC COMPARISON OF TWO MODIFIED METHODS OF HEPATOCYTE DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Li, Rong - *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
Zhao, Yang - *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
Yourick, Jeffrey - *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
Sprando, Robert - *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*
Gao, Xiugong - *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA*

Abstract: Directed differentiation of human induced pluripotent stem cells (iPSCs) into hepatocytes could provide an unlimited source of liver cells, and therefore holds great promise for regenerative medicine, disease modeling, drug screening, and toxicology studies. Various methods have been established during the past decade to differentiate human iPSCs into hepatocyte-like cells (HLCs) using growth factors or small molecules. However, direct comparison of the differentiation efficiency and the quality of the final HLCs between different methods has rarely been reported. In the current study, we devised two hepatocyte differentiation methods, namely Method 1 and Method 2, through modifying existing well-known hepatocyte differentiation strategies, and compared the resultant cells phenotypically and functionally at different stages of hepatocyte differentiation. Higher differentiation efficiency and reproducibility were observed from Method 2, which generated highly homogeneous functional HLCs at the end of the differentiation process. The cells exhibited morphology closely resembling primary human hepatocytes (PHHs) and expressed high levels of hepatic protein markers. More importantly, these HLCs demonstrated several essential characteristics of mature hepatocytes, including major serum protein (albumin, fibronectin, and alpha-1 antitrypsin) secretion, urea release, glycogen storage, and inducible cytochrome P450 activity. Further transcriptomic comparison of the HLCs derived from the two methods identified 1,481 differentially expressed genes (DEGs); 290 gene ontology (GO) terms in the biological process (BP) category were enriched by these genes, which were further categorized into 34 functional classes. Pathway analysis of the DEGs identified several signaling pathways closely involved in hepatocyte differentiation of pluripotent stem cells, including signaling pathways regulating pluripotency of stem cells, Wnt signaling pathway, TGF-beta signaling pathway, and PI3K-Akt signaling pathway. These results may provide a molecular basis for the differences observed between the two differentiation methods and suggest ways to further improve hepatocyte differentiation in order to obtain more mature HLCs for biomedical applications.

Keywords: induced pluripotent stem cells, hepatocyte differentiation, hepatocyte-like cells

TOPIC: MUSCULOSKELETAL

936

MODELING LIMB MORPHOGENESIS BASED ON POSITIONAL INFORMATIONS IN ORGANOID CULTURE IN MOUSE AND HUMAN

Tsutsumi, Rio - *ASHBi, Kyoto University, Kyoto, Japan*
Eiraku, Mototsugu - *Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan*

Abstract: Approximately 1 in 2000 infants are bone with Congenital limb deficiencies. Although developmental genetic studies have contributed to our detailed understanding on molecular mechanisms which control limb morphogenesis, in vitro model of 3-dimensional limb morphogenesis has not yet been established. Limb skeletons are derived from limb bud mesenchymal cells during embryonic development. Shapes the limb skeletons are different depending on their positions in the limb. For example, stylopod (upper arm and thigh) has one bones, zeugopod (lower arm and shin) has two bones, and autopod (hand, foot) has many small bones including phalanges. After formation of limb skeletons, those bone grow differently depending on their position. For example, human thigh and shins grow relatively longer than arms. This pattern of differential bone growth is one of the specific features of bipedal human body as extant non-human apes shows longer arms than legs. In order to establish in vitro model of position-specific cartilage morphogenesis and growth, we isolated limb bud mesenchyme from mouse embryo and differentiate those cells into limb cartilage in 3-dimensional culture. We showed that the shapes and number of cartilage can be manipulated by positional information provided by morphogens, and the number of cells. Next, we aimed to establish a model of human limb morphogenesis in 3-dimensional culture. We drove differentiation of human embryonic stem cells towards limb bud mesenchyme. Interestingly, bud structure expressing limb mesenchyme marker genes came out of the cellular aggregates, which is reminiscent of limb bud initiation during embryonic development. By culturing those cells in the conditions equivalent to the mouse in vitro model, we are currently developing methods to engineer human limb morphogenesis in vitro. We expect that our novel in vitro models would provide unique opportunities to study human-specific limb morphogenesis and limb congenital deficiencies.

Funding Source: This research is supported by Japan Society for the Promotion of Science and Japan Science and Technology Agency.
Keywords: Limb development, organoids, morphogenesis

TOPIC: NEURAL

938

PATIENT-DERIVED STEM CELLS TO STUDY THE PATHOLOGY OF AUTISM SPECTRUM DISORDERS-RELATED VOLTAGE-GATED CALCIUM CHANNEL GAIN-OF-FUNCTION MUTATIONS

Tisch, Marcel - *Genomics, Stem Cell Biology and Regenerative Medicine, University of Innsbruck, Austria*
De Mingo Alemany, María Carmen - *Pediatric Endocrinology Unit, Hospital Universitario la Fe, Valencia, Spain*
Ortner, Nadine - *Pharmacology and Toxicology, University of Innsbruck, Austria*
Geisler, Stefanie - *Pharmacology and Toxicology, University of Innsbruck, Austria*
Lechner, Miriam - *Genomics, Stem Cell Biology and Regenerative Medicine, University of Innsbruck, Austria*
Fauth, Christine - *Human Genetics, Medical University of Innsbruck,Austria*
Suarez-Cubero, Marta - *Genomics, Stem Cell Biology and Regenerative Medicine, University of Innsbruck, Austria*
Török, Ferenc - *Pharmacology and Toxicology, University of Innsbruck, Austria*
Günther, Katharina - *Genomics, Stem Cell Biology and Regenerative Medicine, University of Innsbruck, Austria*
Defrancesco, Michaela - *Psychiatry I, Medical University of Innsbruck, Austria*
Striessnig, Jörg - *Pharmacology and Toxicology, University of Innsbruck, Austria*
Edenhofer, Frank - *Genomics, Stem Cell Biology and Regenerative Medicine, University of Innsbruck, Austria*

Abstract: Voltage-gated calcium channels (VGCCs) are involved in many physiological processes and are highly expressed in human cardiac, endocrine and brain tissues. In addition, increasing evidence emerges that VGCC are key modulators of early neurodevelopment. VGCC gain-of-function mutations, as observed in the Cav1.3 encoding CACNA1D gene, have been linked to a range of neurological pathologies, including Autism Spectrum Disorders (ASD). One such mutation affects the Cav1.3 L271 residue, which is highly conserved among VGCC pore-forming α1-subunits. Electrophysiological studies in tSA-201 cells overexpressing Cav1.3 L271H indicate that this mutation induces channel gain of function by lowering the voltage dependency of channel activation and inactivation, thereby permitting increased subthreshold inward Ca2+ currents. However, currently no functional studies are available on how this mutation affects early neurodevelopment or the physiology of disease-relevant human neurons. Here, we describe the generation of an induced pluripotent stem cell (iPSC)-line, carrying the heterozygous Cav1.3 L271H mutation, through reprogramming of peripheral blood mononuclear cells (PBMC) obtained from a patient diagnosed with a severe neurodevelopmental disorder. By employing Sendai virus OSKM vectors, we have generated stable iPSC lines expressing pluripotency markers. The obtained Cav1.3-mutant lines can be differentiated into all three germ layers and show a normal karyotype. Additionally, a neural progenitor cell (NPC) line, expressing NPC markers SOX2, NESTIN and PAX6, has been generated. We demonstrate that these NPCs express Cav1.3, as confirmed by RT-qPCR, and that these cells can readily be used for in vitro differentiation into neurons typically associated with abnormal Cav1.3 activity, such as dopaminergic neurons. We will present a comprehensive analysis including immunostainings, electrophysiological recordings and calcium imaging aimed to investigate how the Cav1.3 L271H mutation interferes with neural differentiation and neuronal function. Overall, this study will broaden our knowledge regarding the role of Cav1.3 channels during neurodevelopment and their pathogenic role in CACNA1D channelopathies, thereby paving the way for novel therapeutic strategies for affected individuals.

Funding Source: FWF-P35722 (Jörg Striessnig) FWF SFB F7810 (Frank Edenhofer) PhD School “Ageing and Regeneration” (Marcel Tisch)
Keywords: Voltage-gated calcium channel gain-of-function, Disease modelling, Patient-derived iPSC and NSC

940

MICROTUBULE DEFECTS CAUSED BY ELEVATED LEVELS AND PATHOLOGICAL CHANGES IN TAU AS A POTENTIAL CONTRIBUTOR TO AXONAL DEGENERATION IN TAUOPATHTY

Sun, Xiaohuan - *Neurology and Anatomy, Drexel University, Philadelphia, PA, USA*
Celeste, Karch - *Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA*
Qiang, Liang - *Neurology and Anatomy, Drexel University, Philadelphia, PA, USA*
Baas, Peter - *Neurology and Anatomy, Drexel University, Philadelphia, PA, USA*

Abstract: In tauopathy, tau loses association with microtubules (MTs) in the axon and forms neurofibrillary tangles. Tau is one of the most abundant MT-associated proteins in the axon, but surprisingly little is known about how pathological tau impacts the properties of MTs. Because pathological tau binds less avidly to them, the conventional view has been that the levels and stability of axonal MTs are diminished as a result of the pathology. However, this idea is based on the dogma of tau as a MT stabilizer, which our recent studies on cultured rat neurons have called into question. Those studies suggested that tau actually maintains portions of axonal MTs as dynamic by competing with genuine MT-stabilizers such as MAP6. The situation is further complicated in the case of human disease by the fact that neurons express a very different profile of tau isoforms as they mature, with different isoforms having different MT-binding affinities and propensities for aggregation. To investigate this matter in a more disease-relevant scenario, we used forebrain glutamatergic neurons derived from isogenic human-induced pluripotent stem cells, including those bearing the FTD tauP301S mutation. Significant elevation in the levels of both immature and mature tau isoforms in tauP301S-neurons at different developmental stages was identified when compared with their isogenic wild-type controls. Similar results were also obtained from other tau mutant hiPSC-neurons by other groups. Moreover, this elevation was accompanied by increased phosphorylation of tau, revealed by tau-AT8 staining; as well as the appearance of pathological conformations of tau, revealed by tau-TNT2. These changes coincided with morphological, immunocytochemical, electrophysiological changes, and neurodegenerative phenotypes. Interestingly, the increased tau levels in tauP301S-neurons were associated with increased MT dynamics and reduced MAP6 levels, which is consistent with our thinking on how tau impacts the MT network. In all, these results suggest that disease-relevant tau mutations might present an “overexpression of tau” phenotype at the early pathological stage. Therefore, we set forth to further scrutinize how elevated tau phosphorylation and other pathological conformation changes in tau impact MT properties in various stages of the disease.

Funding Source: This study is funded by Dr. Qiang’s Lisa Dean Moseley foundation grant and Dr. Baas’s FTD DOD grant.
Keywords: Tau, Microtubule, MAP6

942

IDENTIFICATION OF ADENO-ASSOCIATED VIRUS VARIANTS FOR GENE TRANSFER INTO HUMAN NEURAL CELL TYPES BY PARALLEL CAPSID SCREENING

Flitsch, Lea J. - *Institute of Reconstructive Neurobiology, University of Bonn, Germany*
Börner, Kathleen - *Center for Infectious Diseases, Heidelberg University, Heidelberg, Germany*
Stüllein, Christian - *Stüllein Software Engineering (SSE), Riedstadt, Germany*
Ziegler, Simon - *KINSYS GmbH, Karlsruhe, Germany*
Sonntag-Buck, Vera - *Center for Infectious Diseases, Heidelberg University, Heidelberg, Germany*
Semkova, Vesselina - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Au Yeung, Christina - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Schlee, Julia - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Hajo, Mohamad - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*
Mathews, Mona - *LIFE & BRAIN GmbH, Bonn, Germany*
Grimm, Dirk - *Center for Infectious Diseases, Heidelberg University, Heidelberg, Germany*
Brüstle, Oliver - *Institute of Reconstructive Neurobiology, University Hospital Bonn, Germany*

Abstract: Human brain cells generated by in vitro cell programming provide exciting prospects for disease modeling, drug discovery and cell therapy. These applications frequently require efficient and clinically compliant tools for genetic modification of the cells. Recombinant Adeno-associated viruses (AAVs) fulfill these prerequisites for a number of reasons, including the availability of a myriad of AAV capsid variants with distinct cell type specificity (also called tropism). Here, we harnessed a customizable parallel screening approach to assess a panel of natural or synthetic AAV capsid variants for their efficacy in lineage-related human neural cell types. We identified common lead candidates suited for the transduction of directly converted, early-stage induced neural stem cells (iNSCs), induced pluripotent stem cell (iPSC)-derived later-stage, radial glia-like neural progenitors, as well as differentiated astrocytic and mixed neuroglial cultures. We then selected a subset of these candidates for functional validation in iNSCs and iPSC-derived astrocytes, using shRNA-induced downregulation of the citrate transporter SLC25A1 and overexpression of the transcription factor NGN2 for proofs-of-concept. Our study provides a comparative overview of the susceptibility of different human cell programming-derived brain cell types to AAV transduction and a critical discussion of the assets and limitations of the specific AAV capsid screening approach.

Funding Source: German Research Foundation (no. 240245660, no. 272983813, EXC81); German Center for Infection Research (BMBF, TTU-HIV 04.815); European Union’s Horizon 2020 (no. 874758); National Institutes of Health, USA (no. R01 NS100514).
Keywords: AAV screening, Neural cells, Gene transfer

944

GENERATION OF LOW-IMMUNOGENIC HUMAN IPSCS-DERIVED DOPAMINERGIC NEURONS FOR THE APPLICATION IN PARKINSON'S DISEASE MODEL

Chuang, Jen-Hua - *Laboratory of Translational Medicine, Development Center for Biotechnology, Taipei City, Taiwan*
Tung, Ying Tsen - *Laboratory of Translational Medicine, Department Center for Biotechnology, Taipei, Taiwan*
Chou, Feng Cheng - *Laboratory of Translational Medicine, Department Center for Biotechnology, Taipei, Taiwan*
Lu, Ching Yi - *Laboratory of Translational Medicine, Department Center for Biotechnology, Taipei, Taiwan*
Hsieh, Chia Ling - *Laboratory of Translational Medicine, Department Center for Biotechnology, Taipei, Taiwan*

Abstract: Parkion's disease (PD) is a neuro-degenerative disorder. Approximately 1million patients are diagnosed as PD in Taiwan. The main cause is the progressive degeneration or even death of dopaminergic neuron in the substantia nigra that leads to movement impairment. Although the supplementation of dopamine can improve motor functions, when the effects of drugs wear off, the patients would become in poor motor status, whereas increasing medicine dose would induce dyskinesias. It's necessary to develop dopaminergic cell therapy as the potential therapeutic strategy. Autologous hiPSCs derived dopaminergic neurons transplantation was considered as a promising approach for PD patients. However, whether the differentiated cells from patients are healthy and functional after transplantation is another important issue. The allogeneic grafts differentiated from low-immunogenic hiPSCs of healthy donors could prevent the rejection reaction and provide individuals healthier environment. In our study, we utilized Crispr-cas12 to generate a HLA-I component, Beta 2 Microgloublin (B2M) knockout hiPSCs with a systemic quality control investigation. Based on the critical role of CHIR on inducing progenitors of midbrain, we boosted the CHIR concentration from 0.4 uM to 6 uM to direct the cell fate towards to the midbrain after day 11 of differentiation. The progenitors of dopaminergic (DAP) neurons at day 16 were partially cryopreserved for the following rat transplantation and characterized on day 28 and day 50. The DAP expressed higher than 2000 folds of FoxA2/LMX1A/EN1 on day 16 and TH on day 28. Efficient facilitating dopamine releasing by KCl was also determined. Through these in vitro assessments, we have successfully established and optimized the differentiation protocol of B2M KO-hiPSCs derived dopaminergic neurons. We will further evaluate the in vivo functions of grafted dopaminergic neuron in 6-OHDA injured rat models to monitor the behavior improvement for long-term pattern. Hopefully, our low-immunogenic iPSCs derived dopaminergic neuron could have benefits for PD theapeutics.

Funding Source: The funding number is "111YY323" The topic of this grand is "Development of low-immunogenic dopaminergic neuronal cell therapy for Parkinson's disease"
Keywords: Low-immunogenic human iPSCs, dopaminergic neurons, Parkinson's disease

946

DEVELOPMENT OF HUMAN NEURAL PROGENITOR CELL MODELS AND HIGH THROUGHPUT SMALL MOLECULE SCREENING FOR REACTIVATION OF FMR1 IN FRAGILE X SYNDROME HUMAN NEURAL CELLS

Risgaard, Ryan - *Department of Neuroscience, University of Wisconsin, Madison, WI, USA*
Hunt, Jack - *Waisman Center, University of Wisconsin , Madison, WI, USA*
Li, Meng - *Waisman Center, University of Wisconsin, Madison, WI, USA*
Ananiev, Gene - *Carbone Cancer Center Drug Discovery Core, University of Wisconsin, Madison, WI, USA*
Wildman, Scott - *Carbone Cancer Center Drug Discovery Core, University of Wisconsin, Madison, WI, USA*
Zhang, Fan - *School of Pharmacy, University of Wisconsin, Madison, WI, USA*
Bugni, Tim - *School of Pharmacy, University of Wisconsin, Madison, WI, USA*
Zhao, Xinyu - *Waisman Center, University of Wisconsin, Madison, WI, USA*
Bhattacharyya, Anita - *Waisman Center, University of Wisconsin, Madison, WI, USA*

Abstract: Developments in human stem cell modeling and high-throughput screening present promising opportunities for potential drug therapies of Fragile X Syndrome (FXS). FXS is an X-linked genetic disorder and the most common inherited cause of autism and intellectual disability. The majority of FXS cases are caused by expansion of >200 CGG trinucleotides in the 5' untranslated region of the FMR1 gene. Expansion in this region results in hypermethylation and epigenetic silencing of the FMR1 promoter region with subsequent loss of FMR1 gene protein product. FMR1 protein is an RNA-binding protein that is highly expressed in the human brain and canonically functions by regulating the translation of a large number of target genes. Lack of FMR1 protein in humans results in numerous developmental phenotypes characteristic of FXS, including intellectual disability, attention deficit hyperactivity disorder, limb development abnormalities, and seizures. While normal transcription of the FMR1 gene is prevented in FXS by epigenetic silencing, the FMR1 coding sequence remains intact in human FXS patients. This implies that FMR1 protein can be translated and produced in FXS patients if gene transcription were reactivated. However, traditional animal models of FXS do not reflect the FMR1 silencing mutation characteristic of humans. We generated FXS-patient induced pluripotent stem cells (iPSCs) that recapitulate the hallmark epigenetic pathogenesis of FXS - harboring the full FXS CGG expansion mutation and exhibiting FMR1 CpG hypermethylation with drastically reduced FMR1 mRNA expression. We then developed a high-throughput, fluorescent-based assay utilizing FXS iPSC-derived neural cell lines to conduct a massive, unbiased screen for small molecule activators of the FMR1 gene while simultaneously evaluating potential compound toxicity. We report the screening of over 320,000 unique small molecules for reactivation of FMR1, the largest screen for chemical reactivators of FMR1 published to date. This proof-of-principle methodology and large-scale screen demonstrate the utility of human stem-cell-based methodology for the untargeted discovery of reactivators of the human FMR1

gene that can be applied to FXS and many other genetic and epigenetic disorders.

Keywords: Fragile X Syndrome, Drug Screen, Human pluripotent stem cell

TOPIC: PANCREAS

948

IDENTIFYING OSCILLATING HES1 TARGET GENES IN PANCREATIC PROGENITOR MODELS

Raineri, Silvia - *Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Mjøseng, Heidi - *NNF Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Egeskov-Madsen, Anuska - *NNF Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
de Lichtenberg, Kristian - *NNF Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*
Serup, Palle - *NNF Center for Stem Cell Medicine (reNEW), University of Copenhagen, Denmark*

Abstract: HES1 is a transcriptional repressor controlling gene regulatory networks that, once activated, fine tunes the balance between maintenance of an adequate progenitor pool and the initiation of differentiation. We recently uncovered ultradian oscillations in the expression of Hes1 and its target gene Dll1 in the developing mouse pancreas. Here, we aim to define additional targets of HES1 that are following this oscillatory behavior. By combining bioluminescence assays and RNA-seq time-course experiments on cell populations synchronized for Notch signaling status, we analyze HES1 oscillation patterns and define Notch and HES1 target genes in human ES cell-derived pancreatic progenitors and models of pancreatic duct cells, the facultative adult pancreatic progenitor cells. Intersection of RNA-seq data with our list of bona-fide, direct HES1 targets provides a first step towards elucidating the functional importance of Notch pathway oscillations in human pancreatic development.

Funding Source: reNEW grant number NNF21CC0073729
Keywords: Notch signalling, Pancreas development, Oscillations

TOPIC: PLURIPOTENT STEM CELLS

950

ZNT8 IS DISPENSABLE FOR THE DIFFERENTIATION AND FUNCTION OF HUMAN STEM-CELL DERIVED BETA CELLS

Du, Qian - *Pediatrics Molecular Genetics, Columbia University Medical Center, New York, NY, USA*
Sui, Lina - *Pediatrics, Columbia University Irvine Medical Center, New York, NY, USA*
Romer, Anthony - *Pediatrics, Columbia University Irvine Medical Center, New York, NY, USA*
Su, Qi - *Regeneron Pharmaceuticals, New York, NY, USA*
Chabosseau, Pauline - *Cell Biology, Imperial College, London, UK*
Xin, Yurong - *Regeneron Pharmaceuticals, New York, NY, USA*
Kim, Jinrang - *Regeneron Pharmaceuticals, New York, NY, USA*
González, Bryan - *Pediatrics, Columbia University Irvine Medical Center, New York, NY, USA*
Kleiner, Sandra - *Regeneron Pharmaceuticals, New York, NY, USA*
Rutter, Guy - *Cell Biology, Imperial College, London, UK*
Egli, Dieter - *Pediatrics, Columbia University Irvine Medical Center, New York, NY, USA*

Abstract: A rare truncating p.Arg138* variant (R138X) in zinc transporter 8 (ZnT8) is associated with a 65% reduced risk for type 2 diabetes. To address if ZnT8 is required for beta cell development and function, we derived human pluripotent stem cells carrying this mutation and differentiated them into insulin-producing cells. We found that human pluripotent stem cells with homozygous or heterozygous R138X mutation and the null (KO) mutation have normal efficiency of differentiation towards insulin-producing cells, but these cells were depleted of zinc in insulin secretory granules and presented diffuse insulin granules. Insulin secretion is not compromised by introduced mutation in both R138X and KO human stem cell-derived beta cells (sc-beta cells). The function of sc-beta cells after transplantation is comparable between different genotypes. Therefore, ZnT8 loss of function is dispensable for both differentiation and function of human sc-beta cells; engineered beta cells without ZNT8 may thus be useful in cell replacement strategies to confer a protective effect in the context of type 2 diabetes (T2D).

Funding Source: ADA 1-16-ICTS-029; NIDDK UC4 DK104207; NIH P30 DK26687-36; R01 DK 057846-16; the Weezie family foundation; DERC pilot and feasibility grant; Wellcome Trust Investigator (WT212625/Z/18/Z) Award; MRC Program grant (MR/R022259/1).
Keywords: Diabetes, Beta cell, Stem cell

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: EARLY EMBRYO

502

CLONING OF THE LEOPARD CAT EMBRYOS BY INTERSPECIES SOMATIC CELL NUCLEAR TRANSFER

Tsai, Li-Kuang - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*
Wu, Hong-Ting - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*
Sung, Li-Ying - *Institute of Biotechnology, National Taiwan University, Taipei, Taiwan*

Abstract: Leopard cat (Prionailurus bengalensis), the only wild feline still inhabiting the main island of Taiwan, has been listed as a nationally endangered species since 2017. Somatic cell nuclear transfer (SCNT) offers a potential means for the preservation of leopard cat (L-Cat) genetics. However, the endangered status limits the availability of L-Cat oocytes for SCNT. In this study, we worked to develop an interspecies SCNT (iSCNT) protocol by using domestic cat (D-Cat) oocytes as the recipient cytoplasm. First, the parthenogenetically activated (PA) D-Cat embryo was used as a model to optimize the embryo culture condition and investigate the dynamic profile of pluripotent and trophectoderm (TE) lineages. The D-Cat oocytes were parthenogenetically activated by electroporation combined with cytochalasin B and 6-dimethylaminopurine (6-DMAP) treatment, subsequently cultured, and resulted in 78.26±2.50% cleavage rate and 24.23±2.97% blastocyst

rate. We traced the spatial and temporal expression of pluripotent markers SOX2 and OCT4, and the TE lineage marker CDX2 in these PA D-Cat embryos. SOX2 and OCT4 became detectable from the 16-cell stage, expressed in both the inner cell mass (ICM) and TE cells in the expanded blastocyst stage, but became much concentrated in the ICM than in the TE at the hatching blastocyst stage. In contrast, CDX2 was detected starting in the early blastocyst stage, and by the hatching blastocyst stage, it was strictly expressed only in TE cells. Utilizing the validated activation protocol, we produced D-Cat SCNT embryos and achieved a blastocyst development rate of 17.62±5.61%, with an average blastomere number of 108±22. Lastly, we conducted iSCNT using L-Cat fibroblast and the enucleated D-Cat oocyte. The blastocyst rate of iSCNT was 9.19±4.75% with 114±31 blastocyst cells, which is compatible with those of D-Cat SCNT. Collectively, this study established an efficient iSCNT platform to produce L-Cat cloned embryos that is valuable for conservation of this endangered species.

Funding Source: Ministry of Science and Technology, Taipei, Taiwan, R.O.C. Grant number MOST 109-2313-B-002-003-MY2
Keywords: Felidae family, leopard cat, interspecies SCNT

TOPIC: EPITHELIAL_GUT

504

RETROSPECTIVE ANALYSIS OF ENHANCER ACTIVITY AND TRANSCRIPTOME HISTORY STATE USING THE DCM TIME MACHINE

Van Leeuwen, Marieke E. - *Developmental Biology, Erasmus MC, Amsterdam, Netherlands*
Boers, Ruben - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Boers, Joachim - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Tan, Beatrice - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Wassenaar, Evelyne - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Sleddens, Esther - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Gonzalez Sanchez, Erlantz - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Baarends, Willy - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*
Gribnau, Joost - *Developmental Biology, Erasmus MC, Rotterdam, Netherlands*

Abstract: Cell state changes in development and disease are controlled by gene regulatory networks, the dynamics of which are difficult to track in real time. In this study we use a system which enables whole genome cell state tracing, the DCM time machine (DCM-TM). DCM-TM is a methylation based sequencing system which uses the bacterial methylase DCM. A DCM-RNA polymerase II subunit B (RPB2) fusion protein can add a DCM methylation label (DML) to the gene body of active genes and enhancers at induction. DML does not affect gene transcription and is propagated during S phase enabling lineage tracing. We applied DCM-TM technology to study intestinal homeostasis, following enterocyte differentiation back in time. Our analysis indicated that DCM-TM not only labels active genes but also active enhancers. By pulse labelling using doxycycline we generated maps of gene and enhancer activity throughout differentiation from intestinal stem cells (ISC) towards enterocytes, indicating rapid and simultaneous activation of enhancers and nearby genes. Our study provides new insights in the absorptive-secretory lineage decision in ISC differentiation, and shows that ISCs retain a unique chromatin landscape required to maintain ISC identity and delineate future expression of differentiation associated genes. DCM-TM has wide applicability in tracking cell states, providing new insights in the regulatory networks underlying cell state changes in development and differentiation. In future studies DCM-TM will be used to study gene regulation of all intestinal epithelial cells in homeostasis and regeneration.

Funding Source: Oncode institute
Keywords: Lineage tracing in the epithelium of the small intestine, Epigenetic labeling, enhancer and gene regulatory networks.

TOPIC: HEMATOPOIETIC SYSTEM

506

CELLULAR BARCODING REVEALS SELECTIVE EXPANSION OF MESENCHYMAL STROMAL CELL CLONES CAUSED BY ACUTE LEUKEMIA

Baron, Chloé - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Avagyan, Serine - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Potluri, Rajiv - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
McKenna, Aaron - *Molecular and Systems Biology, Dartmouth University, Hanover, NH, USA*
Yang, Song - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Zon, Leonard - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*

Abstract: Hematopoietic stem cells (HSCs) reside niches that provide regulatory signals for their function and are affected in leukemia. Mutation of the MYC oncogene family is a frequent event leading to leukemogenesis. We developed a new zebrafish model of acute erythroid leukemia (AEL) by overexpression of human CMYC under the blood specific promotor draculin (drl). Analyses of drl:CMYC marrows demonstrated a significant expansion of progenitors and a decrease of erythroid, lymphoid and myeloid mature cells (fc= 4.8, -4.5, -3.3, -6.5; $p < 0.000001$). RNA-Sequencing of drl:CMYC marrows revealed an upregulation of the erythroid master regulator gata1a (fc= 1.4, $p = 0.01$) and fetal hemoglobins hbbe1.1/2 (fc = 4.7, 2.9; $p = 0.0004$). Primary and secondary transplantation of drl:CMYC marrows resulted in engraftment and disease propagation (7/7; 17/18). We used GESTALT to uniquely barcode single cells using CRISPR-CAS9 during embryonic development. We induced a round of barcoding at the one-cell stage and another one at 28 hours post-fertilization, the time of HSC birth. We injected these GESTALT embryos with drl:CMYC to induce AEL, barcode HSCs and their niche to perform clone tracing. The number of HSC clones was decreased by half compared to controls ($p = 0.008$) indicative of a clonal expansion of the disease. We performed barcode and single-cell transcriptome profiling of flk1:GFP+ niche endothelial cells and found no significant change in the number of endothelial cells clones. However, we identified a novel AEL venous endothelial population upregulating 99 genes (fc > 1; $p < 0.05$), including apelin, cxcr4a and angpt2a suggestive of angiogenesis likely supporting leukemogene-

sis. We sorted cxcl12a:dsRed+ niche stromal cells and found that AEL marrows have significantly less stromal clones (fc = -2.1, p = 0.02) that are selectively amplified (>20% of the stromal compartment). We hypothesized that AEL expands a subset of stromal cells to promote disease progression and scRNA-Seq of 3,263 cxcl12a:dsRed+ stromal cells revealed an increased fraction of lepr+ mesenchymal stromal cells (MSCs, 66 vs 24% in controls). Together our data support a model in which leukemia induces a remodeling of the HSC niche, specifically a selective clonal amplification of a transcriptionally distinct subset of MSCs, to promote disease progression.

Funding Source: Human Frontier Science Program Organization: long term postdoctoral fellowship LT000494/2020-L
Keywords: Hematopoietic stem cell niche, Acute Leukemia, Mesenchymal Stromal Cells

TOPIC: IMMUNE SYSTEM

508

T-CELL REJUVENATION: A NOVEL APPROACH TO PARTIALLY REPROGRAMMING T CELLS TO IMPROVE THEIR IMMUNOTHERAPEUTIC PROPERTIES

Vizcardo, Raul - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Yamazaki, Yasuhiro - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Maeda, Takuya - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Tamaoki, Naritaka - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Huang, Yin - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Fiovaranti, Jessica - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Kutlu, Burak - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Potluri, Shobha - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*
Restifo, Nicholas - *T Cell Rejuvenation Team, Lyell Immunopharma, South San Francisco, CA, USA*

Abstract: Key parameters have been identified affecting T-cell function over the lifespan of an organism: age and identity. Increased age and differentiation of T cells are associated with decreased efficacy and reduced benefit of immunotherapy in solid tumors. While T-cell clonotypes can be renewed from a stem cell-like state, the T cell's capacity for self-renewal, proliferation, persistence, and antitumor activity is adversely impacted by aging. Others have developed methods to de-differentiate cells into induced pluripotent stem cells (iPSCs) that return to embryonic immaturity and lose functional identity. However, T-cell re-differentiation into the desired functional phenotype from iPSCs is a process that is complex and time-consuming. We sought to counter the impact of aging on T-cell function through our cellular rejuvenation technology. We partially reprogrammed aged T cells by transiently expressing transcription factors associated with iPSC reprogramming, allowing reprogrammed T cells to re-acquire the phenotype and function of conventional T cells while exhibiting a younger epigenetic age. Our data is the first to illustrate the ability to partially "turn back" the epigenetic clock of a T cell without a return to a pluripotent state, as seen with complete iPSC reprogramming. The resulting T cells do not require complex re-differentiation steps, thus reducing the time required for reprogramming and differentiation. These rejuvenated T cells are characterized by improved cell growth and biomarker expression associated with T-cell stemness. In vitro studies revealed that rejuvenated NY-ESO-1-targeted T-cell receptor (TCR) or CD19-targeted chimeric antigen receptor (CAR) T cells exhibit improved antitumor properties compared with unrejuvenated T-cell controls in sequential cell killing assays. We also found that partial rejuvenation can be applied to tumor infiltrating lymphocytes (TIL), resulting in similar effects on T-cell stemness phenotype and improved cell growth, indicating potential application across several T-cell therapy modalities. Through partial reprogramming, our T-cell rejuvenation technology has the potential to efficiently improve the function of T-cell immunotherapies and improve outcomes of patients with solid tumors.

Funding Source: Lyell Immunopharma
Keywords: T Cell Rejuvenation, Cancer Immunotherapy, Adoptive Cell Therapy

TOPIC: MUSCULOSKELETAL

510

OPTIMIZATION OF BOVINE MUSCLE-DERIVED STEM CELL CULTURE AND MAINTENANCE OF CHARACTERISTICS

Lee, Eun Ji - *Stem Cell, MKbiotech, Daejeon, Korea*
Kim, Eun Young - *Stem Cell, MKbiotech, Daejeon, Korea*
Kim, Ryeong Eun - *Stem Cell, MKbiotech, Daejeon, Korea*
Kim, Min Kyu - *MKbiotech, MKbiotech, Daejeon, Korea*

Abstract: Cultured meat produced from livestock-derived muscle stem cells is in the spotlight for the supply of meat due to the food shortage problem caused by the increase in the population. This study aimed to establish bovine muscle-derived stem cell lines (bM-SCs) and maintain myogenic characteristics in vitro culture, a basic study for producing beef culture meat. To maintain the characteristics of bM-SCs during in vitro culture, skeletal muscle cell growth media including growth factors, dexamethasone, fetuin, and calf serum was used to culture the bM-SCs, and it could lead to a stable proliferation without morphology change. As a result of evaluating the differentiation ability and muscle stem cell characteristics of established bM-SCs, it was confirmed that tri-lineage differentiation was possible and positive on PAX7 (muscle satellite cell marker), CD29 and CD56 (muscle stem cell marker). In addition, only cells representing myogenic (+CD56/+CD29/-CD31/-CD45) were sorted and purified by excluding non-myogenic cells from primary cells isolated from muscle tissue using the magnetic activated cell sorting (MACS). And the sorted cell treated 100nM rapamycin for 24 hours to maintain proliferation capacity and muscle stem cell characteristics. Cells treated with rapamycin maintained an undifferentiated state and showed persistent expression of PAX7 compared to the control. In conclusion, myogenic cells purified through MACS maintained their characteristics as muscle stem cells consistently through rapamycin treatment. This provides insights on establishing cattle muscle stem cell lines to produce cultured meat, a future resource, and optimizing culture methods.

Funding Source: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry through High Value-added Food Technology Development Program, funded

by Ministry of Agriculture, Food and Rural Affairs (321025-05).
Keywords: Muscle-derived stem cell, Cultured meat, Myogenic

TOPIC: NEURAL

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HIGHLY PARALLELIZED, MULTIPARAMETRIC MEASUREMENTS OF SYNAPTIC TRANSMISSION FOR CNS THERAPEUTIC DISCOVERY USING HUMAN IPS CELL-DERIVED NEURONAL MODELS

Williams, Luis A. - *Cell Biology, Q-State Biosciences Inc., Cambridge, MA, USA*
Fink, James - *Cell Biology, Q-State, Cambridge, MA, USA*
Rajendran, Sakthi - *Cell Bio, Q-State, Cambridge, MA, USA*
Borja, Gabriel - *Engineering, Q-State, Cambridge, MA, USA*
Dhandapani, Aishwarya - *Cell Bio, Q-State, Cambridge, MA, USA*
Pichler, Monika - *Analytics, Q-State, Cambridge, MA, USA*
Mohan, Arvind - *Engineering, Q-State, Cambridge, MA, USA*
Harikrishnan, Karthiayani - *Cell Biology, Q-State, Cambridge, MA, USA*
Meier, Joshua - *Cell Bio, Q-State, Cambridge, MA, USA*
Grooms, Jennifer - *Imaging, Q-State, Cambridge, MA, USA*
Jacques, Jane - *Imaging, Q-State, Cambridge, MA, USA*
McManus, Owen - *Research and Development, Q-State, Cambridge, MA, USA*
Gerber, David - *Research and Development, Q-State, Cambridge, MA, USA*
Dempsey, Graham - *Research and Development, Q-State, Cambridge, MA, USA*

Abstract: The full realization of next generation CNS therapeutics will require the input of vast, high quality data sets directly linked to fundamental human biology with a deeper understanding of phenotypes resulting from pathological perturbations at the level of individual neurons and synapses. Here we report a new approach to measuring synaptic transmission in tens of thousands of individual hiPSC-neurons per day in 96-well plate format using all-optical electrophysiology (OptopatchTM). Our system allows for fine-tuning of pre- vs. post-synaptic neuronal populations through the orthogonal expression of CheRiff, a voltage actuator that enables action potential stimulation in pre-synaptic neurons, and QuasAr, a voltage reporter that enables fluorescent readout of post-synaptic potentials. Our analytics framework then computes a multi-dimensional disease phenotype based on changes in key synaptic parameters. We identified factors that greatly improved (>2x) the synaptic maturity and signal of hiPSC-neuronal cultures, and synaptic measurements from 11,325 individual neurons showed appropriate dose-response reduction in excitatory postsynaptic potential (EPSP) amplitude and area with AMPAR and NMDAR blockade via NBQX and MK801 treatment, respectively. We applied our approach to disease modeling in hiPSC-neurons through large-scale phenotyping of CRISPR/Cas9-edited iPSC lines with genetic disruption of genes associated with neurodevelopmental disorders including STXBP1, MECP2, SHANK3 and SYNGAP1. Synaptic phenotyping of 10-30 cell lines in each case identified distinct multiparametric functional changes. For example, in neurons lacking MECP2 protein, whose loss-of-function causes Rett Syndrome, lack of MECP2 resulted in reduced EPSP area and fewer synaptically active cells (n>10,000 neurons measured). In neurons with disruption of the pre-synaptic protein STXBP1, whose haploinsufficiency causes Developmental and Epileptic Encephalopathy 4, loss of STXBP1 resulted in complete loss of synaptic transmission (n>100,000 STXBP1-/- neurons measured across 13 iPSC lines). The discovered synaptic phenotypes and preliminary data in the context of drug discovery across diverse therapeutic modalities such as small molecules, antisense oligonucleotides, and gene therapy will be presented.

Keywords: human iPSC-neurons, all optical electrophysiology, disease modeling and drug discovery

TOPIC: NT - GENERAL

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STRAIGHT-IN: A PLATFORM FOR HIGH-THROUGHPUT TARGETING OF LARGE DNA PAYLOADS IN HUMAN PLURIPOTENT STEM CELLS

Blanch Asensio, Albert - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Brandão, Karina - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Davis, Richard - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
de Korte, Tessa - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Grandela, Catarina - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Mol, Mervyn - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Mummery, Christine - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
van Meer, Berend - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*
Yiangou, Loukia - *Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands*

Abstract: Inserting large DNA payloads (>10 kb) into specific genomic sites of mammalian cells remains challenging. Applications ranging from synthetic biology to evaluating the pathogenicity of disease-associated variants for precision medicine initiatives would greatly benefit from tools that facilitate this process. We have merged the strengths of different classes of site-specific recombinases and combined these with CRISPR/Cas9-mediated homologous recombination to develop a platform for stringent site-specific replacement of genomic fragments >50 kb in size in human induced pluripotent stem cells (hiPSCs). This platform, termed STRAIGHT-IN (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation), enables a genomic locus to be repeatedly modified not only rapidly but also efficiently. We demonstrate the versatility of STRAIGHT-IN by: (i) inserting various combinations of fluorescent reporters into hiPSCs to assess excitation-contraction coupling cascade in derivative cardiomyocytes, and; (ii) simultaneously targeting multiple variants associated with an inherited cardiac arrhythmic disorder into a pool of hiPSCs. Furthermore, we confirmed the expected electrophysiological phenotype was observed for one of the variants introduced. In summary, STRAIGHT-IN offers a precise approach to generate panels of hiPSC lines containing either multiple combinations of transgenes or potential disease variants in the same cell line and genomic context both efficiently and cost-effectively.

Funding Source: Novo Nordisk Foundation (NNF21CC0073729) Starting Grant (638030) and a Proof of Concept grant (885469) from the European Research Council VIDI fellowship (91715303) Netherlands Organ-on-Chip Initiative (024.003.001)

Keywords: site-specific recombination, targeted gene modification, Bxb1 integrase, Cre recombinase, CRISPR/Cas9, human pluripotent stem cells, disease modeling, cardiomyocyte, synthetic biology, synthetic gene circuit

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ORGANOID SCAFFOLD WITH FIBRILLAR COLLAGEN, LAMININ, AND HYALURONIC ACID

Mizuno, Kazunori - *Nippi Research Institute of Biomatrix, Nippi, Inc., Toride, Japan*
Sasaki, Takako - *School of Medicine, Oita University, Oita, Japan*
Takemura, Yukitoshi - *Research and Development, Kyo Diagnostics, Kyoto, Japan*
Fujita, Kazumasa - *Nippi Research Institute of Biomatrix, Nippi, Inc., Toride, Japan*
Kumazawa, Yuki - *Nippi Research Institute of Biomatrix, Nippi, Inc., Toride, Japan*
Murasawa, Yusuke - *Nippi Research Institute of Biomatrix, Nippi, Inc., Toride, Japan*

Abstract: Three-dimensional culture is essential for mimicking real human tissues and organs. Currently, mouse basement membrane extract (BME), or mouse Engelbreth-Holm-Swarm (EHS) tumor derived material is widely used as a scaffold. Although this gel has been used for a variety of applications, the cells often do not exhibit tissue structure and function as in vivo. In order to develop a new substrate that is closer to living organisms, we combined collagens, hyaluronic acid, and laminin E8 fragments. We tried to find conditions in which they would remain in solution at low temperatures and gel at the temperature of the cell culture. The basic formula was porcine skin collagen I/III, recombinant human laminin-511 E8 fragment, and hyaluronic acid. In addition, collagen (acid soluble, pepsin extracted; I&III, IV, V) and laminin E8 fragment isoforms (111, 332, & 511) were used to adjust the tissue-specific environment. When the cells isolated from kidney, colon, and liver derived of the 12-day mouse embryos are cultured in this gel, cells were differentiated and organized. When mouse embryonic-derived renal cells were cultured in the gel, the cells were monodispersed immediately after culture, but aggregation of the cells was observed after one day of culture, and self-assembly into tissues with branching ureter was advanced by Day 7 of culture. Embryonic-derived renal cells not only form cell aggregates, but also the cell aggregates bound to each other with type IV collagen basal ECM, ureter branching, vascular network, renal glomerular formation, and nephron progenitors. In the developmental hepatocyte culture, the expression of hepatoblast maintenance, bile duct formation, and angioplasty was confirmed. As a result of patient derived colorectal cancer cell culture, it was confirmed that organized cancer cells induced. Our gel's concept is to build physiological connective tissue ECM including basement membrane and interstitial collagen fibrils. This new gel can be used as an alternative to BME, but it also has the potential to overcome the shortcomings of BME. It has more versatile properties that can regulate both mesenchymal and epithelial tissues in the body.

Keywords: collagen, extracellular matrix, 3D scaffold

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ENGINEERING EXTRACELLULAR VESICLES OF HUMAN MESENCHYMAL STEM CELLS THROUGH METABOLIC ALTERATIONS IN A NOVEL VERTICAL WHEEL BIOREACTOR

Li, Yan - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*
Jeske, Richard - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*
Sun, Li - *Biomedical Sciences, Florida State University, Tallahassee, FL, USA*
Liu, Chang - *Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL, USA*
Duke, Leanne - *Biomedical Sciences, Florida State University, Tallahassee, FL, USA*
Jung, Sunghoon - *Process Development, PBS Biotech, Camarillo, CA, USA*
Meckes, David - *Biomedical Sciences, Florida State University, Tallahassee, FL, USA*

Abstract: Human mesenchymal stem cells (hMSCs) are mechanically sensitive undergoing phenotypic changes when subjected to shear stress encountered in dynamic bioreactor cultures. However, little has been known how shear stress affects the secretion and cargo profiles of hMSC-extracellular vesicles (EVs) including the subset, "exosomes", which contain therapeutic proteins, nucleic acids, and lipids from the parent cells. In this study, bone marrow–derived hMSCs were expanded on Synthemax II microcarriers in the novel PBS mini 0.1L vertical wheel bioreactor system under variable shear stress at 25 rpm, 40 rpm, and 64 rpm (0.1-0.3 dyn/cm2). The results show up to a 20-fold increase in cell densities. The bioreactor system promotes EV secretion by 2.5-fold and upregulates the expression of EV biogenesis markers, including the endosomal sorting complexes required for transport (ESCRT)-dependent ALIX, TSG101, and HRS by 2-6 fold, as well as ESCRT-independent SMAD 2/3 by 3-8 fold, Rab27a/b by 2-4 fold, and glycolysis genes (e.g., PDK1, HK2, by 2-8 fold), compared to the static 2D culture. Higher exosomal markers CD63 and CD81 was observed for bioreactor EVs than the static 2D EVs, with similar expression of HSC70 and syntenin-1 based on Western blot. The microRNA cargo was also promoted in the EVs from bioreactor culture including miR-10 (by 15-20 fold), miR-19a and 19b (by ~5 fold), 21 (by 20-50 fold), miR-132 (by 20-40 fold), and miR-377 (by 20-40 fold). The protein cargo was characterized by proteomics analysis, showing the bioreactor culture resulted in most of the differentially expression proteins. The functional assay of the isolated EVs was shown in an in vitro wound healing model and T cell proliferation assay. In addition, the scalability of the PBS vertical wheel bioreactor system was demonstrated in 0.5L bioreactor, showing similar or better hMSC-EV secretion and cargo content compared to the 0.1L bioreactor. This study advances our understanding of bio-manufacturing of stem cell-derived EVs for applications in cell-free therapy towards treating neurological disorders such as ischemic stroke, Alzheimer's disease, and multiple sclerosis.

Funding Source: National Science Foundation (1743426)
Keywords: extracellular vesicles, human mesenchymal stem cells, vertical wheel bioreactor

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CREATION OF BLADDER ASSEMBLOIDS MIMICKING TISSUE REGENERATION AND CANCER

Kim, Eunjee - *Biological Sciences, Seoul National University, Seoul, Korea*
Choi, Seoyoung - *Biological Sciences, Seoul National University, Seoul, Korea*
Lee, Juhee - *Biological Sciences, Seoul National University, Seoul, Korea*
Kim, Yunhee - *Biological Sciences, Seoul National University, Seoul, Korea*
Hong, Soojung - *Biological Sciences, Seoul National University, Seoul, Korea*
Shin, Kunyoo - *Biological Sciences, Seoul National University, Seoul, Korea*

Abstract: Current organoid models are limited by their inability to mimic mature organ architecture and associated tissue microenvironments. Here we create multilayer bladder 'assembloids' by reconstituting tissue stem cells with stromal components to represent an organized architecture with an epithelium surrounding stroma and an outer muscle layer. These assembloids exhibit characteristics of mature adult bladders in cell composition and gene expression at the single-cell transcriptome level,and recapitulate in vivo tissue dynamics of regenerative responses to injury. We also develop malignant counterpart tumour assembloids to recapitulate the in vivo pathophysiological features of urothelial carcinoma. Using the genetically manipulated tumour-assembloid platform, we identify tumoural FOXA1, induced by stromal bone morphogenetic protein (BMP), as a master pioneer factor that drives enhancer reprogramming for the determination of tumour phenotype, suggesting the importance of the FOXA1–BMP–hedgehog signalling feedback axis between tumour and stroma in the control of tumour plasticity.

Keywords: Assembloid, Tissue stroma/microenvironment, Organoid

TOPIC: PLURIPOTENT STEM CELLS

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XENOBARRIER IN HUMAN-MOUSE INTERSPIECES CHIMERA FORMATION

Masaki, Hideki - *Institute of Medical Science, The University of Tokyo, Bunkyo-ku, Japan*
Nakauchi, Hiromitsu - *Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA*

Abstract: Human->animal interspecies chimera formation is quite useful in terms of generating functional human organ/tissue/cells from human pluripotent stem cells (PSCs) through developmental process. We used mouse pre-implantation embryos as a host and sought the condition enabling human->mouse interspecies chimera formation. Previously, we reported that anti-apoptotic factor, such as BCL2, -overexpressing rodent primed PSCs could form chimeras with mouse pre-implantation embryos despite developmental stage gap. BCL2-overespressing human primed PSCs also formed chimeras with mouse embryos, however, survived human progenies did not integrate into surrounding host animal tissues but autonomously differentiated. To understand the reason of abnormal development of BCL2-overexpressing human progenies, we analyzed their distribution in various developmental stages. When BCL2-overexpressing human primed PSCs were injected into mouse morulae, the cells temporally localized in mouse ICM or pre-implantation epiblast, however, none of their progenies localized in mouse post-implantation epiblast but localized in extraembryonic region during E6.5-E7.5. Epiblast cells differentiate into three-germ layers through gastrulation stimulated by the signaling molecules secreted from surrounding tissues. Therefore, failure to localize into post-implantation epiblast would explain abnormal development in later stages, though it is not sure how the cells localized in extraembryonic region in gastrula move to embryo body at later stages. In consistent to in vivo observation, mixed cultured human and mouse primed PSCs showed segregation within short time of period. These findings indicate that localization of engrafted human PSCs into mouse post-implantation epiblast is required for proper differentiation of human progenies and integrated development of human-mouse interspecies chimeras.

Keywords: regenerative medicine, pluripotent stem cells, organ transplantation

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SAFETY OF CELL THERAPY PRODUCTS: IN VITRO METHODS TO ASSESS THE TUMORIGENICITY OF HUMAN CELL-BASED THERAPEUTIC PRODUCTS

Henry, Marianne - *Technology, Process and Innovation, Cell and Gene Therapy Catapult, London*
Marginean, Dragos - *Technology, Process and Innovation, Cell and Gene Therapy Catapult, London, UK*
Smart, Matthew - *Technology, Process and Innovation, Cell and Gene Therapy Catapult, London, UK*
Nicholas, Nicole - *Technology, Process and Innovation, Cell and Gene Therapy Catapult, London, UK*
Moss, David - *Technology, Process and Innovation, Cell and Gene Therapy Catapult, London, UK*
Yasuda, Satoshi - *National Institute of Health Sciences, Kawasaki, Japan*
Sato, Yoji - *Kawasaki, National Institute of Health Sciences, Kawasaki, Japan*
Libertini, Silvana - *Novartis, Basel, Switzerland*
Harada, Kousuke - *Takeda, Tokyo, Japan*
Watanabe, Takeshi - *Takeda, Tokyo, Japan*
Bando, Kiyoko - *Sumitomo Dainippon Pharma, Osaka, Japan*
Terai, Orie - *Sumitomo Dainippon Pharma, Osaka, Japan*
Chen, Connie - *Health and Environmental Sciences Institute, Washington, DC, USA*
Pereira Mouriès, Lucilia - *Health and Environmental Sciences Institute, Washington, DC, USA*

Abstract: Human pluripotent stem cells (hPSC) have the potential to revolutionise regenerative medicine. However, there are concerns associated with hES/iPSC-derived products, in particular, the possibility of residual undifferentiated PSCs persisting in the final product, which could lead to tumorigenicity. Currently, there is no globally accepted consensus on the evaluation of methods for tumorigenicity in-vivo or in-vitro. This results in a high variability of data presented in regulatory submissions and difficulty in interpretations. To address this challenge, the Health and Environmental Sciences Institute (HESI) Cell Therapy - TRAcking, Circulation, & Safety (CT-TRACS) committee, convened international experts in the field, from multiple sectors and geographic areas, to critically review currently available in-vivo and in-vitro testing

methods for tumorigenicity evaluation against expectations in international regulatory guidelines. The outcome of this effort was recently published in a position paper, which highlighted that the establishment of robust methods, internationally harmonised for tumorigenicity assessment of cell therapy products, is critically important, not only for product developers, but also for regulatory authorities and patients. Here, we will present the follow-up activity of the committee: the launch of an international collaborative project to evaluate in-vitro testing methods focusing on the detection of residual undifferentiated PSCs using ddPCR. The two objectives, among many, for the multi-site study are to develop better, standardised in-vitro models for predicting tumorigenicity and to aid researchers, developers and regulators to assess the safety of products with more confidence and contribute to faster/earlier decision-making.

Keywords: Tumorigenicity, Regulatory Issues, Safety

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DEFINING CULTURE: COST-EFFECTIVE MEDIUM TO IMPROVE STEM CELL REPRODUCIBILITY

Schlagal, Caitlin R. - *Research and Development, Defined Bioscience, Inc., San Diego, CA, USA*
Lyra-Leite, Davi - *Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*
Burridge, Paul - *Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*
Rees, Steven - *CEO, Defined Bioscience, Inc., San Diego, CA, USA*
Karpiak, Jerome - *Research and Development, Defined Bioscience, Inc., San Diego, CA, USA*

Abstract: Biological variation in animal-derived products results in cell culture media that are difficult to use in academic work, as well as at scale for industrial and clinical uses. For example, inherent variability in animal serum can lead to the extra burdens of batch testing and bulk acquisition that are expensive and unsustainable for a long-term stable product. Our goal is to discover, define, and distribute reagents and media that provide precision and consistency, reduce research costs based on irreproducible work, and are amenable to Current Good Manufacturing Practice (cGMP) consistent with FDA regulations for materials used in therapeutic production. In this domain, we previously developed and commercialized HiDef-B8 with hyper-stable FGF2-G3 using existing technologies as substrates to improve stem cell culture performance. Building on our previous success with HiDef-B8, we applied engineering principles, specifically semi-empirical 3-factor rotatable central composite design of experiments (DOE), to further optimize combinatorial concentrations of NRG1, insulin, and FGF2. We report growth rate and surface protein expression as our indicators of pluripotency, verified by metabolic assay and flow cytometry. Data from these two discretely measurable parameters were entered into the statistical 3D model to drive our next DOE iteration. Next, we verify pluripotency via downstream outcome for cardiomyocyte, neural, and endothelial differentiation. Defined Bioscience is motivated to apply these new findings, adapting our current processes, to further reduce manufacturing costs to meet requirements of the otherwise unaffordable large scales needed for emerging technologies such as iPSC-derived therapeutics and the growing cultivated meat space. Collectively, our data confirm that we have an opportunity to further advance stem cell medium for a broader market. Our ongoing goals are to decrease cost of scale, increase efficiency, and facilitate wider accessibility of defined media and components.

Funding Source: This work was supported by NIH NIGMS grant R44 GM140750.
Keywords: Cell culture, Defined media, DOE optimization

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A NOVEL VITRONECTIN VARIANT SUPPORTS IMPROVED EXPANSION OF PLURIPOTENT STEM CELLS UNDER ANIMAL-FREE CONDITIONS

Flynn, Kevin C. - *Stem Cell & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Andersen, Marnelle - *Stem Cell & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Galitz, David - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Jones, Bryan - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Lu, Xi - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Naqvi, Tahmina - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Otto, Neil - *GES, Bio-Techne, Minneapolis, MN, USA*
Perr, Julliana - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*
Peterson, Brittni - *Stem Cells & Gene Therapy, Bio-Techne, Minneapolis, MN, USA*

Abstract: An essential aspect of harnessing the potential of pluripotent stem cells (PSCs) and their derivatives for regenerative medicine is the development of animal free and chemically defined conditions for ex vivo cultivation. PSCs including embryonic and induced PSCs (iPSCs) are in the early stages of clinical trials for various indications including degenerative diseases and traumatic injury. A key step in the workflows generating these cells for more widespread clinical use is their safe and robust ex vivo cultivation. This entails optimization of cell culture media and substrates that are safe and consistent while maintaining robust functionality. Here we describe the design of human vitronectin (hVTN) variants with improved manufacturability in a bacterial expression system and improved function in comparison to wild-type VTN or other polypeptide fragments previously characterized. In conjunction with an animal component free media formulation, these hVTN fragments provide animal free conditions for the enhanced expansion of iPSCs. Furthermore, we show that these iPSCs can be efficiently differentiated into the three major germ layers and cortical neurons, thereby closing the loop on a completely defined, animal free workflow for cell types relevant for regenerative medicine.

Keywords: Animal-Free, ex vivo, expansion

TOPIC: EARLY EMBRYO

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A HUMAN PLURIPOTENT STEM CELL-BASED HIGH-THROUGHPUT PLATEFORM WITH ARTIFICIAL INTELLIGENCE TECHNOLOGY TO SCREEN FOR DEVELOPMENTAL TOXICANTS

Chen, Yichang - *Mechanistic Toxicology Branch, NIEHS/NTP, Durham, NC, USA*
Birla, Shama - *Mechanistic toxicology Branch, NIEHS/NTP, Durham, NC, USA*
Tokar, Erik - *Mechanistic toxicology Branch, NIEHS/NTP, Durham, NC, USA*

Abstract: Environmental factor-induced birth defects raise the risk for lifelong disabilities to survivors and increase the economic burden to their families and society. While over 80,000 chemicals are 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 to humans is desired. Here, we developed a human pluripotent stem cell (hPSC)-based high-throughput platform with artificial intelligence technology to screen for developmental toxicants. Embryoid bodies (EBs) generated from hPSCs were used since their formation recapitulates early embryogenic processes. A two-part toxicity prediction system was built upon the transcriptional response and morphological change of EBs to 35 chemicals with confirmed teratogenicity in humans and experimental animals. The expression change of 20 hallmark genes of embryogenesis was subjected to machine learning with different algorithms. The Random Forest-based classification model showed a good accuracy (53%) to categorize the 35 chemicals correctly into four different risk levels, forming the first part of the prediction system. The second part of the system is based on chemical-elicited structural alterations in EBs, captured by high-content fluorescent imaging with germ layer-specific markers. A highly accurate (81%) prediction model based on deep learning technology was obtained through training with 31,644 EB images. To validate the prediction accuracy and prove the practical value of this screening system, the teratogenicity of an additional 20 chemicals with limited toxicity information was assessed by this platform and the results were consistent with previous studies. Tretinoin, as an example, was classified as a 'high' risk teratogen to humans by both subordinary prediction models and showed a 34% toxicity similarity with Etretinate, a confirmed teratogen from the same vitamin A derivative family. Together, these results present a promising capability of our screening platform in identifying human developmental toxicants and understanding their etiology.

Keywords: Embryonic bodies, Teratogen, Deep learning

~~TOPIC: IMMUNE SYSTEM~~

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~~ASSESSING CYTOTOXICITY OF HUMAN IPSC-DERIVED NATURAL KILLER CELLS~~

~~**Lu, Xi** - *Stem Cell Research and Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Goetz, Christine - *Antibody Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Peng, Li-Jen - *Antibody Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Perr, Juliana - *Stem Cell Research and Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Degese, Maria - *Stem Cell Research and Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Bonnevier, Jody - *Antibody Development, Bio-Techne, Minneapolis, MN, USA*~~
~~Flynn, Kevin - *Stem Cell Research and Development, Bio-Techne, Minneapolis, MN, USA*~~

~~Abstract: Natural killer (NKs) cells serve as the basis for next generation immune therapies due to their ability to target and eliminate tumor cells without prior sensitization. NK based immune therapies also show less occurrence of cytokine release syndrome and graft versus host disease. However, isolating and expanding large numbers of peripheral blood NK (PB-NK) cells for therapeutic development is problematic as they represent only 5-15% of circulating lymphocytes. Expanding and differentiating human induced pluripotent stem cells (h iPSC) to NKs provide an alternative and more attractive source of NKs for creating allogeneic "off the shelf" cell therapy. We expanded h iPSCs under animal free conditions and differentiated them to NK (iNK) using a xeno-free process. The iNK cells were subsequently expanded under feeder free conditions. Using a flow cytometry panel, these iNKs were compared to peripheral blood derived NK cells. The iNK cells were CD56+/CD3- and displayed NK activation markers such as NKG2D, NKp30, and NKp46. Additionally, the effector functions of iNK and PB-NKs were evaluated and compared. These assays include Granzyme B and Perforin degranulation, cytotoxicity and tumor killing as assessed via a flow based killing assay and live cell imaging. We designed an antibody dependent (ADCC) killing assay of different tumor cells (Ramos and SKOV3) to further compare and evaluate iNK and peripheral blood NK. Our results indicate the utility and advantage of differentiating human iPSC to NK cells and establishing methods for evaluating their phenotype and effector functions.~~

~~**Keywords:** Immunology, iNK, ADCC~~

TOPIC: NEURAL

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BRIDGING THE ELECTRODE-NEURON GAP OF A COCHLEAR IMPLANT USING HUMAN PLURIPOTENT STEM CELL-DERIVED AUDITORY NEURONS

Norton, Benjamin M. - *Otolaryngology - Head & Neck Surgery, Northwestern University, Oak Park, IL, USA*
Nella, Kevin - *Mechanical Engineering, Northwestern University, Chicago, IL, USA*
Best, Jonathan - *Research & Development, Cell Guidance Systems, Cambridge, UK*
Matsuoka, Akihiro - *Otolaryngology - Head & Neck Surgery, Northwestern University, Chicago, IL, USA*

Abstract: The cochlear implant (CI), which provides functional restoration in patients with sensorineural hearing loss, forms a neuro-electronic interface with the peripheral auditory nervous system. CI technology functions by electrically stimulating the extant population of auditory neurons (i.e., spiral ganglion neurons [SGNs]). Although CI technology has conferred partial hearing restoration to this patient population over the last few decades, persistent challenges remain including the deciphering of a rich acoustic signal into an electrical pulse-train signal. Central to

these challenges, the "electrode-neuron gap" poses a significant obstacle to advancing past the current plateau in CI performance. In this study, we hypothesized that human pluripotent stem cell (hPSC)-derived SGNs exhibiting directed neurite outgrowth can bridge the gap. hPSC-derived otic neuronal progenitors (ONPs) generated according to our pre-established protocol were seeded into an XC450 microfluidic chip (Xona Microfluidics, Inc., Research Triangle Park, NC, USA). POlyhedrin Delivery System (PODS®; Cell Guidance Systems, Cambridge, UK)-brain-derived neurotrophic factor (BDNF) co-crystals, which release BDNF at a sustained rate, were added to the microfluidic chip to generate a neurotrophic concentration gradient and consequently facilitate differentiation toward the SGN phenotype and direct neurite extension. Three-dimensional diffusion was modeled graphically prior to experimentation. Immunocytochemistry was performed to assess neuronal differentiation and visualize neurites for downstream analysis. Results indicate that SGN-like cells expressed neuronal markers, neurites exhibited directional extension toward PODS®-BDNF co-crystals, and neurites grew in length, all as a function of BDNF concentration. Thus, three-dimensional diffusion modeling in combination with the sustained release of BDNF may allow for controllable, directed neurite outgrowth in vivo. The application of this technology needs to be tested in an animal model as a next step toward clinical realization.

Keywords: cochlear implant, spiral ganglion neuron, inner ear regeneration

TOPIC: NT - GENERAL

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NON-INVASIVE CELL IDENTIFICATION SYSTEM USING RAMAN SPECTROSCOPY

Akagi, Yuka - *Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan*
Kida, Yasuyuki - *Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan*

Abstract: Raman scattering light obtained from cells indicates the distribution and amount of intracellular molecules such as proteins and lipids, which enables the non-invasive and non-staining determination of the state of the cell. However, cell-derived Raman signals are weak and susceptible to fluctuations caused by intracellular structures. To perform label-free classification of single cells using Raman signals, a technique to obtain Raman spectra from a wide area of cells with high sensitivity is necessary. In this study, we developed a Paint Raman Express Spectroscopic System (PRESS), which irradiates a laser beam over a wide area of cells via a fast oscillating two-axis Galvano mirror. This system can acquire molecular information of a single cell in a few seconds, and the obtained spectral information can be used for machine learning to identify the type and state of the cell. In this research, two types of cell identification were verified. First, PRESS enables to obtain spectra from various types of cells such as pluripotent stem cells and human iPS cell-derived neurons, and it was confirmed that each cell type can be classified with high accuracy by applying machine learning. Second, Raman spectra were obtained from human T cells stimulated with CD3/CD28 antibodies to verify the discrimination between pre- and post-activation. Despite the similarity in morphology, PRESS could classify the different activation states with 96% accuracy. In addition, it was possible to predict the activation state of cells with unknown activation state by machine learning. Therefore, PRESS can acquire Raman spectra from a wide range of cells at high speed and can discriminate cell types and activation and differentiation states with high accuracy by applying machine learning. This technology is capable of analyzing single cells without staining and is expected to be applied to cell quality control systems in cell therapy in the future.

Keywords: Raman spectroscopy, Cell identification, Machine learning

TOPIC: PLURIPOTENT STEM CELLS

960

OFF-THE-SHELF NATURAL KILLER CELLS DERIVED FROM HIPSC VIA GENETIC MODIFICATIONS

Yang, Luhan - *Qihan Biotech, Qihan Biotech, Hangzhou, China*
Hu, Jiabiao - *QihanBio, QihanBio, Hangzhou, China*
He, Xiangjun - *QihanBio, QihanBio, Hangzhou, China*
Zhou, Yixuan - *QihanBio, QihanBio, Hangzhou, China*
Le, Lei - *QihanBio, QihanBio, Hangzhou, China*
Xu, Jing - *QihanBio, QihanBio, Hangzhou, China*
yue, Yanan - *QihanBio, QihanBio, Hangzhou, China*
Gao, Yangbin - *QihanBio, QihanBio, Hangzhou, China*
Church, George - *Human Genetics, Harvard, Boston, MA, USA*

Abstract: Human induced pluripotent stem cells (hiPSC)-derived allogeneic Natural Killer (NK) cells are emerging as a promising off-the-shelf cell therapy for cancer. However, host-versus-graft rejection limits the allogeneic NK cell durability in patients, especially when the drug is applied in a redosing regimen. Numerous efforts have been applied to engineer "hypoimmunogenic" immune cells, with limited clinical success. Therefore, a novel genetic combination remains to be developed and validated in a relevant preclinical model to warrant clinical success. In this study, we first established a Non-Human-Primate system for allogeneic NK transfusion and rejection. We then engineered a small library of monkey iPSCs, each one carrying 3-21 genetic modifications. Subsequently, we differentiated engineered monkey iPSCs into NK cells and conducted in vivo screening. We identified that QH-X001 had significantly longer persistency in the allogenic monkey recipient, compared with 3-edit NK cells (MHC1 KO, MHC2 KO, CD47 KI) as the control. We then applied the edits on GMP-grade human iPSCs and demonstrated that QH-X001 does not impact NK differentiation or function, and has robust resistance towards human T cells, NK cells, and complement challenges in vitro. Taken together, we identified that the novel genetic combinations of QH-X001 could render iPSCs-derived NK cells hypoimmunogenic in vivo. We anticipate that this could be a useful asset to greatly enhance cell therapy efficacy.

Keywords: Human induced pluripotent stem cells (hiPSC), NK cells, hypoimmunity

TOPIC: NEURAL

976

DUAL-COLOR CALCIUM IMAGING OPERATES AS A NOVEL DRUG SCREENING PLATFORM IN IPSC-DERIVED HETEROGENOUS NEURONAL CULTURE SYSTEMS

Teaney, Nicole A. – *Research Assistant, Neurobiology, The Rosamund Stone Zander Translational Neuroscience Center (RSZ TNC) - The F.M. Kirby Neurobiology Center – Harvard Medical School – Boston Children's Hospital*

Abstract: Calcium imaging serves as a functional assay to measure single-cell neuronal activity and the connectivity of the neuronal networks. In neurological disorders where neuronal signaling and network activity are altered, calcium imaging in 2D and 3D in vitro models may provide further insight into understanding the underlying cellular mechanisms driving the disorders. Furthermore, calcium imaging can serve as a drug screening platform. While multi-electrode arrays provide critical insight into the overall electrical activity of a complex heterogenous cell culture system, there is low spatial resolution in the readout. To our knowledge, there have been no methods developed to measure the functional activity of a heterogenous population of cell culture system at a single-cell resolution. In the present study, we demonstrate the feasibility of measuring the functional activity of two distinct populations of neurons derived from induced pluripotent stem cells (iPSCs) in a 2D co-culture system in a method described as dual-color calcium imaging. The method includes co-culturing NGN2 and iGABA control iPSC-derived neurons after transducing each population with a separate genetic encoded calcium indicator (GECI). A GFP-based and an mRuby-based GECI are specifically chosen so that the excitation/emission spectra did not overlap with one another (excitation/emission of hSynI-GCaMP6s: 485/510 nm; hSyn1-jRCaMP1b: 550/600nm). Calcium imaging recordings are collected to measure the changes in functional activity of the iGABA and NGN2 neurons before, during, and after the addition of synaptic blockers. As a proof of concept, synaptic blockers are added to the co-culture system to elicit changes in neuronal activity in each neuron population within the co-culture. Such synaptic blockers include Vigabatrin, an irreversible inhibitor of GABA transaminase; CNQX, a competitive AMPA receptor antagonist; and DAP-V, a competitive NMDA receptor antagonist. The use of distinct GECIs in a heterogeneous cell culture system provides a novel platform for single-cell resolution drug screening. Future work will include utilizing the dual-color calcium imaging screening platform in iPSC-derived disease models in which neuronal signaling and connectivity are altered.

Funding Source: Title: The Contribution of Rapamycin-insensitive Processes to Neurological Symptoms in TSC Project number: W81XWH2110209 PD/PI: Sahin, Mustafa Source of support: Department of Defense 07/01/2021-06/30/2024
Keyword: Dual-color calcium imaging, Drug screening platform, iPSC-derived neurons

TOPIC: NEURAL

980

STEM CELL DERIVED EXTRACELLULAR VESICLES AGAINST NEUROINFLAMMATION IN THE FELINE MODEL OF SANDHOFF DISEASE

Naskou, Maria - *Department of Pathobiology and Scott-Ritchey Research Center, Auburn University, Auburn, AL, USA*
Cochran, Ann - *Scott-Ritchey Research Center, Auburn University, Auburn, AL, USA*
Martin, Douglas - *Department of Anatomy, Physiology and Pharmacology and Scott-Ritchey Research Center, Auburn University, Auburn, AL, USA*

Abstract: Sandhoff disease (SD) is a lysosomal storage disease, caused by mutations of the β subunit of N-acetyl-β-hexosaminidase (Hex), that leads to progressive neurodegeneration, muscular paralysis, and cherry-red spots in the macula of eye. Even though favorable results have been demonstrated following administration of adeno-associated viral (AAV) gene therapy in the feline SD model, growing evidence suggests that persistence of neuroinflammation exacerbates the disease outcome. Extracellular Vesicles derived from stem cells (SC-EVs) have been shown to suppress neuroinflammation by exhibiting an anti-inflammatory effect in various models, including Alzheimer's disease, ischemic stroke and traumatic brain injury. However, the function of SC-EVs is dependent on the status of the MSCs from which they derive. There is evidence suggesting that pre-conditioning of MSCs with inflammatory mediators enhances the secretion of SC-EVs that are enriched in anti-inflammatory RNAs and neuroprotective proteins. Our objective was to a) isolate and phenotypically characterize Mesenchymal Stem Cell derived EVs (MSC-EVs) from Bone Marrow and Wharton's Jelly under the influence of different culture conditions and b) evaluate their functionality in ex vivo isolated feline stimulated leukocytes. Mesenchymal stem cells were cultured under standard cell culture conditions and once they reached 75% confluency, they were either exposed to Extracellular Vesicles isolation media for 24 hours or culture media containing inflammatory stimulants (TNF-α and IFN-γ) or 5% exosome depleted FBS for 48 hours. Following their isolation, MSC-EVs were added to LPS stimulated feline leukocytes (n=4) for 1 hour. Leukocyte mRNA levels for TNF-α, IL-6 and IL-10 were performed via RT-PCR. Our data revealed that cell culture conditions affect the phenotypic profile of MSC-EVs. A statistically significant decrease in the mean fold change of IL-6 mRNA expression was found following the addition of MSC-EVs from Bone Marrow and Wharton's Jelly to LPS stimulated feline leukocytes. Our data indicate that MSC-EVs have the potential to downregulate inflammatory responses initiated by peripheral leukocytes. Future studies should evaluate the effect of MSC-EVs against immune cells that are mainly involved in CNS neuroinflammation.

Keywords: Stem Cell derived Extracellular Vesicles, Neuroinflammation, Sandhoff disease

TOPIC: EYE AND RETINA

982

GENERATION OF SYNGENEIC IPSC-DERIVED 3D-BIOPRINTED CHOROID/RPE TISSUE FOR REGENERATIVE MEDICINE AND DISEASE MODELING OF OUTER RETINA BLOOD BARRIER

Park, Tea Soon - *NEI, NIH, Bethesda, MD, USA*
Hirday, Rishabh - *NEI, National Institutes of Health, Bethesda, MD, USA*
Ali, Amir - *NEI, National Institutes of Health, Bethesda, MD, USA*
Quinn, Russell - *NEI, National Institutes of Health, Bethesda, MD, USA*
Nguyen, Eric - *NEI, National Institutes of Health, Bethesda, MD, USA*
Bose, Devika - *NEI, National Institutes of Health, Bethesda, MD, USA*
Bharti, Kapil - *NEI, National Institutes of Health, Bethesda, MD, USA*

Abstract: Human induced pluripotent stem cells (iPSC) can provide multiple lineage specific cells for regenerative therapy. Our recent advances in 3D-bioprinting allowed us to produce RPE/choroid tissue with correctly orientated RPE and choriocapillaris. Here, we report generation of 3D-bioprinted choroid and RPE cells with syngeneic iPSC lines for patient-specific ocular therapy and disease modeling. iPSC lines were generated from healthy and retinal degeneration patients (e.g., age-related macular degeneration, AMD), and differentiated into endothelial cells (EC), pericytes, fibroblasts, and retinal pigmented epithelial (RPE) cells. Differentiated cells were purified, expanded and validated by immunophenotyping and functional tests. Capillary formation by iPSC-derived cells was assessed by a hydrogel tubulogenesis assay. 3D-bioprinting was utilized to assemble EC, pericytes and fibroblasts into choroid-like structures on a biodegradable polymer. One week after the choroid 3D bioprint, RPE cells were seeded on the other side of polymer and matured for 4 weeks. Healthy donor retinal-EC, -pericytes, and scleral fibroblasts were used as a primary cell control. All cell components were cryopreserved and thawed prior to functional validation and assembly. iPSC-RPE monolayers exhibited physiological trans-epithelial resistance (TER) and phagocytic ability. Endothelial identity of CD31+ EC was verified by acetylated-Dil-LDL uptake, tube formation, and expression of CD34, CD146 and CD144. Pericytes were differentiated from CD31- cells using TGFb3 and PDGF-bb and confirmed expression of PDGFR-beta, NG2, and CD44. Fibroblasts were differentiated from pericytes and expressed vimentin, connexin43, and collagen-IV. EC, pericytes and fibroblasts assembled into structurally mature tubes in a tubulogenesis assay. PLGA seeded and matured choroid/RPE complex formed proper Bruch's membrane and superior TER value compared to the primary cell sources. Our results show that all four cell components (RPE, EC, pericytes, and fibroblasts) are successfully differentiated from isogenic human iPSC. This study provides the foundation for clinical application of iPSC-derived 3D-tissue, disease modeling (e.g., Choroideremia and AMD) and study model of immune cell reaction in RPE/choroid.

Funding Source: NEI IRP grant ZIA EY000532-08, NEI IRP Grant ZIA EY000542-07, NEI IRP Grant ZIA EY000533-08, Bright Focus Foundation Grant M2020258

Keywords: iPSC differentiation, RPE, 3D bioprint

TOPIC: CARDIAC

984

SIGNIFICANT REDUCTION IN VENTRICULAR TACHYARRHYTHMIA AFTER TRANSPLANTATION OF CARDIOVASCULAR PROGENITORS INTO MYOCARDIAL INFARCTED PIG HEARTS

Yap, Lynn - *CVMD, Duke-NUS Graduate Medical School, Singapore, Singapore*
Chong, Li Yen - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Tan, Clarissa - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Adusumalli, Swarnaseetha - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Seow, Millie - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Guo, Jing - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Cai, Zuhua - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Loo, Sze Jie - *NHRIS, National Heart Center Singapore, Singapore, Singapore*
Lim, Eric - *CVS, National Heart Center Singapore, Singapore, Singapore*
Lath, Narayan - *RADSC, National Heart Center Singapore, Singapore, Singapore*
ye, Lei - *NHRIS, National Heart Center Singapore, Singapore, Singapore*
Petretto, Enrico - *CVMD, Duke-NUS Medical School, Singapore, Singapore*
Tryggvason, Karl - *CVMD, Duke-NUS Medical School, Singapore, Singapore*

Abstract: Cellular therapy could replace damaged heart muscle for regenerative cardiology. We hypothesized that cardiovascular progenitors (CVPs) would electrically synchronized and mature in vivo thus avoiding the occurrence of VTs. In this study, we reported the derivation and applications of hESC-derived CVPs using highly reproducible (R2 > 0.95) laminin-221 differentiation protocol. We permanently ligated the coronary arteries and intramyocardial transplanted 200 million CVPs into pig model. We utilized 10X spatial transcriptomic analysis at 1- and 2-weeks post-transplantation to demonstrate engraftment and viability of the human CVPs into the infarcted heart. Following that, a total of 3 sham, 10 medium control and 10 CVP transplanted pigs were monitored at 4- and 12-weeks post-transplantation. The cells remained viable and proliferated to formed human graft in the infarcted region as indicated by IVIS imaging and histology staining. Heart function was analyzed by magnetic resonance imaging (MRI) and revealed overall improvement in left ventricular ejection fraction by 21.35 3.3 %, which was accompanied by significant improvements in ventricular wall thickness and wall motion, as well as a reduction in infarction size after CVP transplantation as compared to medium control pigs (p-value < 0.05). Electrophysiology analyses revealed electrical propagation between transplanted cells and host tissue CMs. Temporary episodes of VT over a period of 25 days were developed in 4 out of 10 CVP transplanted pigs and 1 pig had persistent VT, while the rest (n = 5) remained in normal sinus rhythm. All ten pigs survived the experiment without any VT-related death. Finally, computerized tomography (CT) scans revealed no tumor formation after 12-weeks transplantation indicating the long-term safety of the treatment. In conclusion, we

reported the generation of an effective, potent, unmodified CVPs and a significant reduction (50 %) in graft-induced VT as compared to studies transplanted with contracting cardiomyocytes. This method may pave the way for cell therapy of myocardial infarction in humans.

Funding Source: This work has been supported in part by grants from the NMRC of Singapore (MOH-STaR18may-0001), Goh Cardiovascular Research (GCR) (Duke-NUS-GCR/2020/0018) award, Tanoto Foundation and 24th NRF (NRF CRP, CRP24-2020-0083) to KT.

Keywords: Regenerative Cardiology, Cardiovascular progenitors, Ventricular tachyarrhythmia

ISSCR Travel Award Recipient

TOPIC: PLURIPOTENT STEM CELLS

986

DOUBLE INHIBITION OF NOTCH AND DOT1L PROMOTES SOMATIC CELL REPROGRAMMING

Morita, Kazuki - *Department of Periodontology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan*
Kawasaki, Tomoyuki - *Center for Regenerative Medicine, National Center for Child Health and Development, Setagaya-ku, Japan*
Sugawara, Tohru - *Center for Regenerative Medicine, National Center for Child Health and Development, Setagaya-ku, Japan*
Hagiwara, Yusuke - *Department of Gastroenterogical Surgery, Hirosaki University, Hirosaki-city, Japan*
Ichida, Justin - *Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA*
Iwata, Takanori - *Department of Periodontology, Tokyo Medical and Dental University, Bunkyo-ku, Japan*
Umezawa, Akihiro - *Center for Regenerative Medicine, National Center for Child Health and Development, Setagaya-ku, Japan*
Akutsu, Hidenori - *Center for Regenerative Medicine, National Center for Child Health and Development, Setagaya-ku, Japan*

Abstract: Human induced pluripotent stem cells (hiPSCs) are generated by reprogramming somatic cells using a defined set of transcription factors. In particular, derivation of autologous iPSCs opens a way to produce patient-specific iPSC lines and expects to contribute to the exploration of cures and causes of diseases, drug screening, and tailor-made regenerative medicine. The reprogramming process is critical to acquire pluripotency and also to be efficient in the successful generation of hiPSCs, however is still not fully understood. In our previous report, pharmacological inhibition of the NOTCH signaling pathway, via N-[N-(3,5-difluorophenacethyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), and the histone H3 lysine 79, methyltransferase disruptor of telomeric silencing 1-like inhibitor (iDOT1L), promoted the generation of hiPSCs from keratinocytes, whereas the mechanisms and the effect of this double inhibition on other types of cells were not investigated. Here, we found that double inhibition by DAPT/iDOT1L markedly increased iPSC colonies generation from human fibroblast cells in normoxia. To investigate the mechanisms, we focused on mesenchymal to epithelial transition (MET) which is induced in the early stage of reprogramming, and evaluated sequential changes of MET-related genes by qPCR array system, and then BMP and TGF-β signaling significantly changed in DAPT/iDOT1L treated group. In this study, we reported the more facilitating and efficient reprogramming method in the normoxia condition. Moreover, we elucidated the part of mechanisms how the reprogramming efficiency increased to focus on sequential changes of MET-related genes.

Funding Source: This work was supported by a Grant in Aid for Scientific Research (A) (20H00550) and the Japan Agency for Medical Research and Development (AMED) (21bk0104089h0003) to HA.
Keywords: Reprogramming, Induced pluripotent stem cell, Mesenchymal to epithelial transition

TOPIC: NEURAL

988

A COMPUTATIONAL FRAMEWORK FOR PREDICTION OF SIGNALING PATHWAYS MEDIATING REPROGRAMMING OF MESENCHYMAL STEM CELLS INTO NEURONS

El-Badri, Nagwa - *Biomedical Science, Zewail City of Science and Technology, October City, Egypt*
Abou-Shanab, Ahmed - *Biomedical Science Program, Zewail City for Science and Technology, Giza, Egypt*
Badr, Eman - *Computational -Biomedical Science Program, Zewail City for Science and Technology, Giza, Egypt*
Samy, Asmaa - *Computational Concentration-Biomedical Science Program, Zewail City for Science and Technology, Giza, Egypt*
Shouman, Shaimaa - *Biomedical Science Program, Zewail City for Science and Technology, Giza, Egypt*

Abstract: Insults to the nervous system have significant health and socioeconomic burden because of the limited regeneration of the damaged nervous tissue. Reprogramming of human adipose-derived mesenchymal stem cells (hADMSCs) into neurons has been proposed as a promising autologous and non-invasive source for neural regeneration. Current reprogramming approaches to produce neuron-like cells from hADMSCs still suffer proper functionality. In this work, we developed a predictive computational workflow for stem cells differentiation using transcriptomic profiles of primary cell samples (FANTOM5) in combination with network and pathway enrichment analyses. Applying our approach to hADMSCs, we identified several significantly enriched pathways involved in neuronal differentiation, including signal transduction, neurotransmitter receptors, and nervous system development. Based on our analysis, we proposed NCAM signalling pathway for neurite outgrowth and CREB1 phosphorylation via NMDA receptor-mediated activation of RAS signalling as candidate pathways that could stimulate neuron differentiation. The proposed NCAM and NMDA pathways in our study introduce a stepwise differentiation approach from hADMSCs specifically into glutamatergic neurons following differentiation into neural stem cells (NSCs). Glutamatergic neurons are a main excitatory in central nervous system (CNS) through NMDA receptors modulating their neuronal plasticity, synaptogenesis, and survival. These results should facilitate the development of more efficient neuronal differentiation protocols for cell-based therapy.

Keywords: MSCs, Neuron, Bioinformatics, reprogramming, MSCs, Neuron, Bioinformatics, reprogramming, MSCs, Neuron, Bioinformatics, reprogramming

TOPIC: CARDIAC

202

SIGNIFICANT REDUCTION IN VENTRICULAR TACHYARRHYTHMIA AFTER TRANSPLANTATION OF CARDIOVASCULAR PROGENITORS INTO MYOCARDIAL INFARCTED PIG HEARTS

Yap, Lynn - *CVMD, Duke-NUS Graduate Medical School, Singapore*
Chong, Li Yen - *CVMD, Duke-NUS Medical School, Singapore*
Tan, Clarissa - *CVMD, Duke-NUS Medical School, Singapore*
Adusumalli, Swarnaseetha - *CVMD, Duke-NUS Medical School, Singapore*
Seow, Millie - *CVMD, Duke-NUS Medical School, Singapore*
Guo, Jing - *CVMD, Duke-NUS Medical School, Singapore*
Cai, Zuhua - *CVMD, Duke-NUS Medical School, Singapore*
Loo, Sze Jie - *NHRIS, National Heart Center, Singapore*
Lim, Eric - *CVS, National Heart Center, Singapore*
Lath, Narayan - *RADSC, National Heart Center, Singapore*
Ye, Lei - *NHRIS, National Heart Center, Singapore*
Petretto, Enrico - *CVMD, Duke-NUS Medical School, Singapore*
Tryggvason, Karl - *CVMD, Duke-NUS Medical School, Singapore*

Abstract: Cellular therapy could replace damaged heart muscle for regenerative cardiology. We hypothesized that cardiovascular progenitors (CVPs) would electrically synchronized and mature in vivo thus avoiding the occurrence of VTs. In this study, we reported the derivation and applications of hESC-derived CVPs using highly reproducible (R2 > 0.95) laminin-221 differentiation protocol. We permanently ligated the coronary arteries and intramyocardial transplanted 200 million CVPs into pig model. We utilized 10X spatial transcriptomic analysis at 1- and 2-weeks post-transplantation to demonstrate engraftment and viability of the human CVPs into the infarcted heart. Following that, a total of 3 sham, 10 medium control and 10 CVP transplanted pigs were monitored at 4- and 12-weeks post-transplantation. The cells remained viable and proliferated to formed human graft in the infarcted region as indicated by IVIS imaging and histology staining. Heart function was analyzed by magnetic resonance imaging (MRI) and revealed overall improvement in left ventricular ejection fraction by 21.35 ± 3.3 %, which was accompanied by significant improvements in ventricular wall thickness and wall motion, as well as a reduction in infarction size after CVP transplantation as compared to medium control pigs (p-value < 0.05). Electrophysiology analyses revealed electrical propagation between transplanted cells and host tissue CMs. Temporary episodes of VT over a period of 25 days were developed in 4 out of 10 CVP transplanted pigs and 1 pig had persistent VT, while the rest (n = 5) remained in normal sinus rhythm. All ten pigs survived the experiment without any VT-related death. Finally, computerized tomography (CT) scans revealed no tumor formation after 12-weeks transplantation indicating the long-term safety of the treatment. In conclusion, we reported the generation of an effective, potent, unmodified CVPs and a significant reduction (50 %) in graft-induced VT as compared to studies transplanted with contracting cardiomyocytes. This method may pave the way for cell therapy of myocardial infarction in humans.

Funding Source: This work has been supported in part by grants from the NMRC of Singapore (MOH-STaR18may-0001), Goh Cardiovascular Research (GCR) (Duke-NUS-GCR/2020/0018) award, Tanoto Foundation and 24th NRF (NRF CRP, CRP24-2020-0083) to KT.

Keywords: Regenerative Cardiology, Cardiovascular progenitors, Ventricular tachyarrhythmia

POSTER SESSION III: EVEN

4:00 PM – 5:00 PM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: ADIPOSE AND CONNECTIVE TISSUE

602

REGULATORY EFFECT OF CELL DERIVED EXTRACELLULAR MATRIX ON ENDOMETRIAL MESENCHYMAL STEM CELLS

Xu, Jingwen - *Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong*
Chan, Rachel W.S. - *Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong*
Chiu, Philip C.N. - *Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong*
Ng, Ernest H.Y. - *Department of Obstetrics and Gynecology, The University of Hong Kong, Hong Kong*
Yeung, William S.B. - *Shenzhen Key Laboratory of Fertility Regulation, The University of Hong Kong Shenzhen Hospital, Shenzhen, China*

Abstract: Thin endometrium is one of the causes of infertility and recurrent pregnancy loss. The remarkable regenerative capability of endometrial stem cells shows promise for the use in regenerative medicine. Perivascular endometrial mesenchymal stem cells (eMSC) can be identified by co-expression of CD146 and CD140b. Characterization of this cell population showed that they can display high clonogenic activity, proliferative potential, and are multipotent. Since these eMSCs (CD140b+CD146+ cells) are pericytes, it's indicated that the perivascular niche is crucial in regulating the stem cell maintenance. The extracellular matrix (ECM) is a niche factor that plays an important role in the regulation of stem cell behaviors. Therefore, the unique properties of the native perivascular niche can influence eMSC. Cell-derived matrix (CDM) obtained by decellularization of in vitro cultured cell sheet can be used to study the effect on eMSC function as it resembles the native composition and organization of in vivo ECM. In our study, CDM was obtained by decellularization of cell sheet of three cell types: primary endometrial stromal cells, human umbilical vein endothelial cells (HUVEC), and human endometrial epithelial-like cells (Ishikawa cells). The CDM properties were characterized using the electron microscopy and analyzed by histological and immunofluorescent staining. To study the effect of CDM on eMSCs, the proliferation activity, phenotypic expression of eMSCs markers, and clonogenicity were analyzed at 5, 7 and 14 days after recellularization. Our results indicate CDM derived from endometrial stromal cells, HUVEC, and Ishikawa cells can significantly promote the proliferation of eMSCs. Additionally, endometrial stromal cells derived CDM helps eMSCs stemness maintenance and clonogenicity. Further evaluation into the ECM components can be used to regulate eMSCs for regenerative medicine.

Funding Source: National Natural Science Foundation of China /Research Grant Council Collaborative Research Scheme (N_HKU732/20)
Keywords: Tissue stem cells, endometrium, regeneration

TOPIC: CARDIAC

604

INDUCED CARDIOMYOCYTE-LADEN 3D PRINTED AUXETIC CARDIAC PATCH FOR HEART REPAIR

Brazhkina, Olga - *Biomedical Engineering, Georgia Institute of Technology/Emory University, Atlanta, GA, USA*
Park, Jeong Hun - *Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA*
Park, Hyun-Ji - *Biomedical Engineering, Emory University, Atlanta, GA, USA*
Hollister, Scott - *Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA*
Davis, Michael - *Biomedical Engineering, Emory University, Atlanta, GA, USA*

Abstract: Myocardial infarction (MI) is one of the largest contributors to cardiovascular disease and reduces the ability of the heart to pump blood. One promising therapeutic approach to address the diminished function is the use of cardiac patches composed of biomaterial substrates and cardiac cells. New designs to cardiac patches, such auxetic geometries that can expand in multiple directions when stretched longitudinally, can have high energy absorption, and can be modified to suit the mechanics of the infarct and surrounding cardiac tissue. These desirable qualities would be an ideal platform to investigate if auxetic patches can provide a supportive environment for cardiomyocyte function. This project developed an auxetic composite cardiac patch that can support induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) function. An auxetic patch (10mm x 10mm) of a missing rib design composed of polycaprolactone with varying thicknesses (0.2, 0.4, 0.6 mm) with adjustable mechanical properties was 3D printed and combined with 10% w/v gelatin methacrylate and differentiated iPSC-CMs at 10 million cells/mL and 100 million cells/mL. After seeding CMs onto the patches, we saw high cell retention (>75%) after 14 days of culture on all patch thicknesses. There was low expression of pluripotent genes (Oct4, Nanog, Sox2) in the patches, showing CM purity at seeding and high expression of CM-specific genes (Cx43, TNNT), confirming CM phenotype. Immunofluorescence staining for cTnT and α-actinin showed presence on all patches. The inclusion of two seeding densities points to enhanced cell-to-cell contacts causing unique clustered 3D spatial organization (>25 nuclei) after 14-day culture within the patches, which is notably absent on day 7 samples. Calcium transients recorded from CMs embedded within the patches showed spontaneous beating for all thicknesses and are comparable to previous literature studies for iPSC-CMs. The designed patches support induced CM viability and function, while having more favorable mechanical properties. Auxetic patterns show promise for new cardiac biomaterial designs. Future studies will investigate using this patch as a platform for CM maturation and examine implantation into an in vivo rat model of chronic MI to assess the regenerative potential and impact on cardiac function.

Funding Source: AHA Grant #916701
Keywords: Cardiomyocytes, cardiac patch, auxetics

TOPIC: EPITHELIAL_GUT

606

ORGANOID AS A REGENERATIVE THERAPY TO TREAT ULCERS IN A PORCINE MODEL OF RADIATION PROCTITIS AND CONSIDERATION FOR FIRST-IN-HUMAN TRIALS

Lee, Kyung Jin - *Research and Development, ORGANOIDSCIENCES, Seongnam-si, Korea*
Park, Jun-hyeok - *Research and Development, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Lim, Taegyu - *Research and Development, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Jeon, Hyemi - *Research and Development, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Song, Ha-young - *Research and Development, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Jo, Suyoung - *BD, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Choi, Woo-hee - *Research and Development, ORGANOIDSCIENCES LTD., Seongnam-si, Korea*
Yoo, Jongman - *ORGANOIDSCIENCES, Seongnam, Korea*

Abstract: Radiation therapy is frequently used in patients with pelvic cancer. While this can be selectively targeting rapidly proliferating cells such as cancer cells and stem cells, it can trigger DNA damages which lead to microvascular injury to the intestinal mucosa, resulting in ischemia, fibrosis, and eventually neovascular lesions. Notwithstanding those current therapeutic interventions look promising based on current data, they are still inadequate to implement changes practically. In radiation proctitis porcine model, the evaluation for the effect of Adult Tissue derived Organoid based Regenerative Medicine of colon organoid (ATORM-C) on ulcers was conducted. Radiation proctitis was induced by high dose of irradiating their recta, followed by endoscopic transplantation of colonic organoids. For clinical application, porcine colonic organoids were cultured in collagen extra cellular matrix. When the porcine organoids were successfully engrafted into damaged mucosa of the irradiated porcine, the reconstruction of intestinal crypt structure was observed. In addition, reduction for both expression of inflammatory cytokines and fibrosis after the treatment with colonic organoids was monitored. As a result, our development of organoid based regenerative medicine, which can regenerate damaged tissue by radiation and inhibit the fibrosis, may provide fundamental and major therapeutic option for refractory ulcers including inflammatory bowel disease and radiation proctitis which are not easily healed with current treatments. Developing the promising therapy for severe ulcers caused by radiation in porcine model allows us to overcome the current limitations for clinical and industrial application of organoids. Here, we share our achievement and experience of challenges for the first in human trials.

Keywords: Regenerative Medicine, First in Human Trials, Proctitics

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ESSENTIAL ROLE OF P53 IN INTESTINAL EPITHELIUM HOMEOSTASIS AND STEM CELL DIFFERENTIATION

Dubey, Amit - *Immunology and Cellular Biology, University of Sherbrooke, QC, Canada*
Gonneaud, Alexis - *Immunology and Cell Biology, University of Sherbrooke, Sherbrooke, QC, Canada*
Giroux, Véronique - *Immunology and Cell Biology, University of Sherbrooke, QC, Canada*

Abstract: Tumor suppressor p53 regulates self-renewal and differentiation in some adult stem cells but its role in intestinal stem cells remains to be fully described. Intestinal epithelium homeostasis is maintained by two main pools of stem cells: Lgr5+ and reserve stem cells. We recently reported that Krt15+ cells also harbor self-renewal, multipotent and regenerative capacities. Therefore, we hypothesize that Trp53 loss specifically in Krt15+ stem cells will perturb epithelial homeostasis and differentiation. We generated Krt15-CrePR1;Trp53fl/fl (Krt15△Trp53) mice and induced Cre recombination by injecting RU486 (PR agonist). Mice were euthanized at different time points after Cre recombination. Two months following Cre recombination, intestinal morphology wasn't severely affected. However, we observed significant changes in organoid cultures especially following Wnt hyperactivation. Indeed, contrarily to control organoids which formed cystic structures following GSK3 inhibition, Trp53-depleted organoids remained mainly budded structures suggesting that differentiation is still occurring. These results correlated with increased expression of Paneth cell-specific genes and decreased expression of EphB2 receptor and its target genes, a pathway important in cell fate. Twelve months following Cre recombination, Trp53 loss specifically in Krt15+ cells led to several morphological alterations in the small intestine. Increased crypt length and villi width was observed in Krt15△Trp53 mice but cell proliferation wasn't affected. However, number of Paneth cells was increased, and aberrant presence of goblet cells was noted in Krt15 Trp53 mice. We also observed increased number of Tuft and goblet cells in the villi of experimental mice. Surprisingly, we observed some crypt cells that expressed goblet and Paneth cell markers and decreased Notch pathway activation, suggesting that Trp53 loss leads to a dysregulation of secretory cell lineages. Brief, our results suggest that loss of Trp53 in Krt15+ stem cells affects intestinal morphology and cell fate decision potentially through Ephrin signaling.

Keywords: Krt15+ intestinal Stem cell, P53, Stem cell fate, Organoid Culture, Stem cell differentiaion, Ephrin signaling, Intestinal organoid culture

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AUTOPHAGIC STATE PROSPECTIVELY IDENTIFIES FACULTATIVE STEM CELLS IN THE INTESTINAL EPITHELIUM

Johnson, Nicolette - *Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Parham, Louis - *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Na, Jeeyoon - *School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA*
Monaghan, Keara - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Kolev, Hannah - *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Klochkova, Alena - *Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA*
Kim, Melissa - *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Danan, Charles - *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Cramer, Zvi - *Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Simon, Lauren - *Division of Gastroenterology, Hepatology, and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
Naughton, Kaitlyn - *Division of Gastroenterology, Hepatology, and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
Adams-Tzivelekidis, Stephanie - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Tian, Yuhua - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Williams, Patrick - *Division of Gastroenterology, Hepatology, and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
Leu, Nicolae - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Sidoli, Simone - *Department of Biochemistry, Albert Einstein College of Medicine, New York, NY, USA*
Whelan, Kelly - *Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA*
Li, Ning - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Lengner, Christopher - *School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA*
Hamilton, Kathryn - *Division of Gastroenterology, Hepatology, and Nutrition, Children's Hospital of Philadelphia, Philadelphia, PA, USA*

Abstract: The intestinal epithelium exhibits a rapid and efficient regenerative response to injury. Emerging evidence supports a model where plasticity of differentiated cells, particularly those in the secretory lineages, contributes to epithelial regeneration upon ablation of injury-sensitive stem cells. We add to this body of literature by demonstrating that facultative stem cells, marked by the Hopx-CreER allele, are enriched for marker genes and proteins of the secretory lineages, including enteroendocrine, Paneth, goblet, and tuft cells. However, such facultative stem cell activity is rare within these secretory populations. We demonstrate this restricted plasticity in enteroendocrine cells using lineage tracing studies from a novel Chga-CreER-tdTomato allele. We further ask if specific functional properties predict facultative stem cell activity. We utilize TRE-H2B-GFP mice to fractionate cells by age (time elapsed since cell cycle exit) and perform ex vivo organoid formation assays with differently aged secretory cell populations. Strikingly, we find that cell age does not correlate with secretory cell plasticity. We next evaluate how autophagic state contributes to facultative stem cell activity within the secretory lineages. We demonstrate that cells with high autophagy have enhanced organoid formation capacity in vitro, regardless of secretory lineage of origin, and that inhibiting autophagic flux impairs this capacity. Lastly, we confirm that autophagy high intestinal epithelial cells are enriched for non-cycling secretory cells, and that cells with high autophagy are protected from DNA-damaging injury. Together, our findings indicate that autophagic status prior to injury

serves as a lineage-agnostic proxy for the prospective identification of facultative stem cells.

Keywords: Facultative stem cell, Regeneration, Autophagy

TOPIC: EPITHELIAL_LUNG

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BRF1-MEDIATED PARACRINE SIGNALLING FROM PITUITARY STEM CELLS IS REQUIRED FOR TERMINAL DIFFERENTIATION OF PITUITARY COMMITTED PROGENITORS

Willis, Thea L. - *Centre for Craniofacial and Regenerative Biology, King's College London, UK*
Manshaei, Saba - *Developmental Biology and Cancer Department, University College London, UK*
Reen, Virinder - *MRC London Institute of Medical Sciences, Imperial College London, UK*
Pallikonda, Husayn - *MRC London Institute of Medical Sciences, Imperial College London, UK*
Birch, Jodie - *MRC London Institute of Medical Sciences, Imperial College London, UK*
Yianni, Val - *Centre for Craniofacial and Regenerative Biology, King's College London, UK*
Lodge, Emily - *Centre for Craniofacial and Regenerative Biology, King's College London, UK*
Withers, Dominic - *MRC London Institute of Medical Sciences, Imperial College London, UK*
Gil, Jesus - *MRC London Institute of Medical Sciences, Imperial College London, UK*
Martinez-Barbera, Juan Pedro - *Developmental Biology and Cancer Department, University College London, UK*
Andoniadou, Cynthia - *Centre for Craniofacial and Regenerative Biology, King's College London, UK*

Abstract: The pituitary gland is a critical endocrine organ regulating multiple essential physiological processes including growth, reproduction, metabolism and the stress response. Hormone-producing pituitary cell lineages are derived from a population of embryonic precursors expressing the transcription factor SOX2. These cells maintain multipotency into early postnatal life, acting as the resident population of pituitary stem cells (PSCs) and contributing to all the endocrine cell lineages. In addition to this direct contribution to pituitary turnover, paracrine signalling from PSCs is necessary for cell proliferation of neighbouring progenitors. It is not known if SOX2+ PSCs are involved in other aspects of neighbouring cell regulation during normal physiology. Utilising scRNA-sequencing of SOX2+ PSCs from Sox2Egfp/+ mouse pituitaries at three postnatal stages from P3 to P56, we show that the SOX2+ PSC population consists of three subgroups (SC1, SC2 and SC3). We reveal that SC1-SC2 express abundant cytokines and secreted factors, suggesting a paracrine function. In contrast SC3, characterised by robust expression of Lef1, is a committing PSC cluster and its presence diminishes with age. We unearth differential and conserved markers of PSC clusters and identify RNA binding factor BRF1 as conserved in one subgroup at all ages. We show that BRF1 is highly expressed in PSCs and that its dysregulation in embryonic pituitary cells results in severe hypopituitarism due to a failure of two distinct lineage-committed progenitors to terminally differentiate into hormone-producing cells. Additionally, there is a significant reduction of the stem cell compartment. The differentiation failure can be rescued in vitro through co-culture of mutant cells with wild-type stem cells, as well as in vivo, in mutant pituitaries where activation of constitutively active BRF1 is restricted to few SOX2+ PSCs in a mosaic manner. Finally, we identify key ligands underlying this differentiation phenotype, and demonstrate a partial restoration of terminal differentiation in the mutant, when cultured in the presence of exogenous ligands. Together, these data indicate the presence of functionally distinct groups of SOX2+ pituitary stem cells and reveal a critical role for PSCs in driving terminal differentiation of endocrine cells.

Funding Source: CLA: MRC (grant MR/T012153/1). JPMB: CRUK (C54322/A27727). TLW: KCL "Cell Therapies and Regenerative Medicine" Four-Year Welcome Trust PhD Training Programme. SM: Boehringer Ingelheim Fonds (PhD Fellowship).
Keywords: Pituitary stem cell, Paracrine signalling, SOX2

TOPIC: EPITHELIAL_SKIN

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IMMUNE REGULATORY PROPERTIES OF MELANOCYTE STEM CELLS FOR POTENTIAL APPLICATION IN VITILIGO REPIGMENTATION THERAPY

Vathsalya, Manchi - *Nitte University Centre for Stem Cell Research and Regenerative Medicine, NITTE (Deemed to be University), Mangalore, India*
Shetty, Shricharith - *Department of Dermatology, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Shetty, Nikhil - *Department of Plastic Surgery, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Yeshwanth, Sunil - *Department of Pathology, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Shetty, Veena - *Nitte University Centre for Stem Cell Research and Regenerative Medicine, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Basavarajappa, Mohana - *Nitte University Centre for Stem Cell Research and Regenerative Medicine, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*
Patil, Prakash - *Central Research Laboratory, K. S. Hegde Medical Academy, Nitte (Deemed to be University), Mangalore, India*

Abstract: In the adult skin epidermis, melanocytes are continuously replaced by a reservoir of melanocyte stem cells (MelSCs) residing in the hair follicle bulge. Due to their ability to self-renew and differentiate into functional melanocytes, they have been considered as a putative source of autologous cells for vitiligo skin repigmentation. However, vitiligo is an autoimmune condition and the immunogenic properties of MelSCs are yet to be explored. Therefore, this study evaluated the immune regulatory properties of MelSCs derived from vitiligo patients as well as control subjects. MelSCs were isolated by explant technique from extracted hair follicles and expanded up to passage 2. Their identity was confirmed by analyzing the molecular expression of pluripotent stem cell markers, such as octamer-binding transcription factor 4 (OCT4), Nanog and SRY-box transcription factor 2 (SOX2) as well as melanocyte lineage markers, such as melanocyte inducing transcription factor (MITF), tyrosinase related protein-1 (TYRP1), PAX3 and dopachrome tautomerase (DCT). Subsequently, peripheral blood mononuclear cells (PBMCs), following incubation in a stimulating cocktail consisting of tumor necrosis factor alpha (TNF-α) and interferon-γ (IFN- γ), were co-cultured

with MelSCs and maintained for 7 days. Levels of pro-inflammatory cytokines, interleukins [(IL)-2, 1β, 17, 22, 12α], TNF-α and IFN-γ as well as anti-inflammatory cytokines, IL-4, 6, 10 and transforming growth factor-β1 (TGF-β1) were estimated at mRNA and protein level by qPCR and ELISA, respectively, against reference controls. Findings showed slightly, but not significantly, higher levels of anti-inflammatory cytokines IL-6, IL-10 and TGF-β1 than pro-inflammatory cytokines IL-2, IL-22 and TNF-α in cultures with MelSCs. T-cell proliferation was also determined by flow cytometric analysis of CD4 and CD8 markers in co-cultures against T-cell cultures without MelSCs. A significant reduction in expression of T-cell markers was observed in co-cultures suggesting positive immunosuppression by MelSCs. The study findings showed the immunophenotype and expression of immunomodulation-related cytokines of MelSCs along with their immune regulatory effects. This will greatly offer the prospects for the use of MelSCs in vitiligo repigmentation therapy.

Funding Source: This work was supported by Nitte (Deemed to be University), Mangaluru, India.
Keywords: Melanocyte stem cells, Immune modulatory, Vitiligo repigmentation therapy

TOPIC: EYE AND RETINA

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GENERATION OF SYNGENEIC IPSC-DERIVED 3D-BIOPRINTED CHOROID/RPE TISSUE FOR REGENERATIVE MEDICINE AND DISEASE MODELING OF OUTER RETINA BLOOD BARRIER

Park, Tea Soon - *NEI, National Institutes of Health, Bethesda, MD, USA*
Hirday, Rishabh - *NEI, National Institutes of Health, Bethesda, MD, USA*
Ali, Amir - *NEI, National Institutes of Health, Bethesda, MD, USA*
Quinn, Russell - *NEI, National Institutes of Health, Bethesda, MD, USA*
Nguyen, Eric - *NEI, National Institutes of Health, Bethesda, MD, USA*
Bose, Devika - *NEI, National Institutes of Health, Bethesda, MD, USA*
Bharti, Kapil - *NEI, National Institutes of Health, Bethesda, MD, USA*

Abstract: Human induced pluripotent stem cells (iPSC) can provide multiple lineage specific cells for regenerative therapy. Our recent advances in 3D-bioprinting allowed us to produce RPE/choroid tissue with correctly orientated RPE and choriocapillaris. Here, we report generation of 3D-bioprinted choroid and RPE cells with syngeneic iPSC lines for patient-specific ocular therapy and disease modeling. iPSC lines were generated from healthy and retinal degeneration patients (e.g., age-related macular degeneration, AMD), and differentiated into endothelial cells (EC), pericytes, fibroblasts, and retinal pigmented epithelial (RPE) cells. Differentiated cells were purified, expanded and validated by immunophenotyping and functional tests. Capillary formation by iPSC-derived cells was assessed by a hydrogel tubulogenesis assay. 3D-bioprinting was utilized to assemble EC, pericytes and fibroblasts into choroid-like structures on a biodegradable polymer. One week after the choroid 3D bioprint, RPE cells were seeded on the other side of polymer and matured for 4 weeks. Healthy donor retinal-EC, -pericytes, and scleral fibroblasts were used as a primary cell control. All cell components were cryopreserved and thawed prior to functional validation and assembly. iPSC-RPE monolayers exhibited physiological trans-epithelial resistance (TER) and phagocytic ability. Endothelial identity of CD31+ EC was verified by acetylated-Dil-LDL uptake, tube formation, and expression of CD34, CD146 and CD144. Pericytes were differentiated from CD31- cells using TGFb3 and PDGF-bb and confirmed expression of PDGFR-beta, NG2, and CD44. Fibroblasts were differentiated from pericytes and expressed vimentin, connexin43, and collagen-IV. EC, pericytes and fibroblasts assembled into structurally mature tubes in a tubulogenesis assay. PLGA seeded and matured choroid/RPE complex formed proper Bruch's membrane and superior TER value compared to the primary cell sources. Our results show that all four cell components (RPE, EC, pericytes, and fibroblasts) are successfully differentiated from isogenic human iPSC. This study provides the foundation for clinical application of iPSC-derived 3D-tissue, disease modeling (e.g., Choroideremia and AMD) and study model of immune cell reaction in RPE/choroid.

Funding Source: NEI IRP grant ZIA EY000532-08, NEI IRP Grant ZIA EY000542-07, NEI IRP Grant ZIA EY000533-08, Bright Focus Foundation Grant M2020258
Keywords: iPSC differentiation, RPE and choroid, 3D bioprint

TOPIC: HEMATOPOIETIC SYSTEM

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RESOLVING EMBRYONIC ORIGINS OF THE BLOOD THROUGH CELLULAR BARCODING

Bowling, Sarah - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
van Leeuwen, Anais - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
de Soysa, Yvanka - *Neurology, Boston Children's Hospital, Boston, MA, USA*
Van Egeren, Debra - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Yu, Qi - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Walker, Alec - *Neurology, Boston Childrens Hospital, Boston, MA, USA*
Walker, Alicia - *Neurology, Boston Childrens Hospital, Boston, MA, USA*
Alcedo, Karel - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Patel, Sachin - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*
Stevens, Beth - *Neurology, Boston Childrens Hospital, Boston, MA, USA*
Gottgens, Berthold - *Wellcome-MRC Cambridge Stem Cell Institute, Cambridge University, Cambridge, UK*
Camargo, Fernando - *Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*

Abstract: Hematopoietic stem cells (HSCs) generated in early development are thought to sustain life-long hematopoiesis, though our understanding of the origins of these cells is still limited. Although it is established that HSCs arise from endothelial cells lining blood vessels of the embryo, there is still important debate about the anatomical site that produces long-lived hematopoietic cells. Progress has been impeded by the lack of tools that enable the labelling and tracking of individual cells in vivo. We have applied our new mouse tool, name CARLIN, to address this question. CARLIN contains expressed, inducible barcodes that enable

high-resolution single cell lineage tracing in vivo. Use of this model has allowed us to trace, at the single cell level, fetal and adult blood cells back to their embryonic endothelial site of origin. Our findings support existing studies demonstrating that myeloid cells generated early in development, including those that give rise to life-long microglia in the brain, are yolk-sac-derived. In contrast, we find that definitive blood progenitors, including HSCs, are derived solely from the aorta and umbilical and vitelline arteries, and have no detectable contribution from the yolk sac or placenta. Furthermore, our data support the existence of a distinct, HSC-independent wave of blood emergence that segregates early in development and gives rise to a subset of life-long tissue resident macrophages and specific 'innate-like' lymphoid cell populations. These cells arise from a lineage of endothelial cells distinct from those that generate definitive HSCs. These data point toward diverse embryonic origins for long-lived blood cells which could shed light the drivers of cellular heterogeneity in adult tissues, as well as inform protocols for productive generation of blood cells from iPSCs in vitro.

Funding Source: European Molecular Biology Organization (EMBO), Wellcome Trust
Keywords: Hematopoiesis, Cellular barcoding, Layered immunity

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FUCOSYLATION IS ABSOLUTELY REQUIRED FOR HUMAN HEMATOPOIETIC STEM CELLS TETHER AND SLING FORMATION AND MIGRATION IN VIVO

Al-Amoodi, Asma - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Li, Yanyan - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Nozue, Shuho - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Habuchi, Satoshi - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*
Merzaban, Jasmeen - *Bioscience, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia*

Abstract: Accumulating evidence demonstrates that abnormal fucosylation expression plays an important role in cell behavior from promoting cancer cell metastasis when it is overexpressed to hindering/impeding adult stem cell migration and engraftment when it is absent or limited. Using human GCSF-mobilized peripheral blood CD34pos hematopoietic stem cells (mPB-CD34pos), we sought to characterize the role of fucosylation on migration and engraftment. Fucosylation was inhibited by culturing mPB-CD34pos cells with 2-fluoro-L-fucose (2FF). Additionally, to assess the impact enhanced fucosylation has, recombinant fucosyltransferase 6 (rhFTVI) was used. Flow cytometric and Western blot analysis clearly illustrated that 2FF treatment dramatically inhibited core fucosylation resulting in low Sialyl Lewis X expression and low binding to E-selectin. To determine the contribution of fucosylation on functional migration of mPB-CD34pos cells, physiological flow-based assays were used including the fluorescence multiplex cell rolling assay and microfluidics-based live-cell fluorescence imaging. Notably, fucosylated cells resulted in an increase in the number of rolling cells with long tight tethers, while cells treated with 2-FF rolled significantly faster with much lower numbers of tethers and slings that were shorter in size. Assessment of mPB-CD34pos engraftment in vivo using NSG mice showed that pretreatment of with 2FF significantly reduced engraftment while FTVI treatment showed an improvement. Beyond selectin binding, the migration towards SDF-1 was measured in a transwell assay and the transmigration was significantly suppressed in cells treated with 2FF while rhFTVI had no significant affect. To evaluate the effect of the treatments on cell-intrinsic parameters, colony formation assay were used and found that mPB-CD34pos maintained their ability to produce differentiated colonies. Moreover, our data indicates that there was no variation observed in gene expression of fucosyltransferases enzymes following either treatment. Overall, our study unveils the critical role of core fucosylation in mPB-CD34pos migration, and that although well-recognized that E-selectin expression on marrow microvessels is crucial to recruitment, the exact involvement of ligands fucosylation is incomplete.

Keywords: Cells homing, Hematopoietic stem cells, Fucosylation, Homing enhancing and inhibition, Rolling imaging, in vivo xenoengraftment NSG

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BNIP3LB REGULATED MITOPHAGY MAINTAINS THE EMBRYONIC POOL OF HEMATOPOIETIC STEM AND PROGENITOR CELLS BY PROTECTING THEM FROM ROS INDUCED APOPTOSIS

Meader, Ellie - *Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Wrighton, Paul - *Division of Genetics, Brigham and Womens Hospital, Boston, MA, USA*
Oderberg, Isaac - *Division of Genetics, Brigham and Womens Hospital, Boston, MA, USA*
Leder, Mindy - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Hachimi, Mariam - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Quenzer, Eleanor - *Division of Genetics, Brigham and Womens Hospital, Boston, MA, USA*
Long, Tim - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Frame, Jenna - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Lim, Sung-Eun - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*
Goessling, Wolfram - *Division of Genetics, Brigham and Womens Hospital, Boston, MA, USA*
North, Trista - *Division of Hematology/Oncology, Boston Childrens Hospital, Boston, MA, USA*

Abstract: We have previously shown that Reactive Oxygen Species (ROS) promote Hematopoietic stem and progenitor cell (HSPC) specification in the dorsal aorta, however in the adult marrow, it is known that HSCs must maintain low levels of ROS to protect the stem cell pool against excessive cycling, differentiation, and loss of stemness. We aim to elucidate the developmental timing and mechanism by which HSCs transition from their initial dependence on ROS, to their precise regulation of it in the adult marrow. Since adult HSCs regulate ROS by maintaining high levels of mitophagy, a process in which damaged mitochondria, which disproportionately generate ROS, are removed and recycled, we sought to define the initiation, regulation and impact of mitophagy in embryonic HSPC development. Our recent data shows that oxidative stress limits HSPC numbers as early as their colonization of the secondary hematopoietic niche, the caudal hematopoietic tissue (the zebrafish analog of the fetal liver); this timepoint coincides precisely with the initiation of mitophagy in

HSCs as visualized by live imaging using a recently developed fluorescent transgenic reporter, Tg(ubi:mitoGR) in the zebrafish embryo. Our scRNAseq and in situ-hybridization analyses show mitophagy is induced via the bnip3lb receptor, a homolog of the NIX gene, which is responsible for developmentally regulated mitophagy in reticulocytes, lens fibers and macrophages, but which, thus far, has no reported role in maintaining HSCs. We determine that reducing mitophagy via morpholino directed knockdown of bnip3lb decreases HSC marker expression in the CHT, whereas chemically or genetically upregulating mitophagy elevates HSC numbers as measured by runx1 and cmyb WISH, and quantified by CD41:GFP HSPC reporter expression. Mechanistically, we found that bnip3lb knockdown increases ROS levels, and that reducing ROS with N-acetyl-cysteine rescues HSPCs. Finally, we demonstrate that the enhancement in ROS levels caused by reduced mitophagy appears to increase apoptosis in the CHT region and alter HSPC fate. We therefore propose that developmentally programmed mitophagy directed by bnip3lb is responsible for protecting proliferative embryonic HSCs from the harmful effects of oxidative stress while expanding the HSC pool to seed lifelong hematopoiesis.

Funding Source: This material is based on work supported by the NIH under grants No: U01HL134812, RC2DK120535, and R01HL154480
Keywords: Mitophagy, HSC, ROS

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SPATIOTEMPORAL MAPPING OF PROGENITOR AND STEM CELL DYSREGULATION AFTER MURINE VOLUMETRIC MUSCLE LOSS

Larouche, Jacqueline A. - *Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA*
Aguilar, Carlos - *Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA*

Abstract: Volumetric muscle loss (VML) incites reductions in muscle function and quality of life, but the cellular and molecular factors that drive the pathological VML response and prevent healing remain poorly understood. As such, quantitative mapping of immune and stem/progenitor cell dysfunction after VML along with spatial mapping of altered intercellular communication is needed. Spatial transcriptomics (spGEX) generates unbiased RNA sequencing (RNA-seq) datasets in a spatially registered manner paired with histological imaging, enabling coupled insights into mechanisms of expression changes with spatial context. We employed spGEX on VML-injured tissues 7 days post injury (dpi) and annotated the tissue into zones of (1) complete muscle loss, (2) a transitional region that partitions the lost muscle from the remaining musculature, and (3) in-tact muscle. Zone 1 was characterized by an abundance of mononucleated cells consistent with previous observations of inflammation, zone 2 was characterized by both mononucleated cells and myofibers with centrally located nuclei, indicating active regeneration or degeneration, and zone 3 contained muscle fibers with peripherally located nuclei. Gene set enrichment analysis yielded enrichments in gene sets associated with the immune system, stress, and defense in zones 1 and 2 while zone 3 contained enriched gene sets for muscle structure development. To increase resolution, we integrated the spGEX datasets with matched single-cell RNA-Seq datasets. In zone 1, we observed signatures for neutrophils, macrophages, and fibro-adipogenic progenitors (FAPs) in several states. In zone 2, muscle stem cells and myonuclei were observed along with inflammatory and progenitor cells. The gene expression signature of zone 3 was predominantly alike that of myonuclei. We confirmed cell localization patterns immunohistologically, including the absence of MuSCs and presence of FAPs in zone 1. Ongoing work is exploring whether antifibrotic treatment modulates this immune-FAP-MuSC patterning and through which cellular and molecular mechanisms. Collectively, this enhances our understanding of the immune cell-progenitor cell-stem cell crosstalk that drives regenerative dysfunction and provides further insight into possible avenues for fibrotic therapy exploration.

Keywords: stem-cell niche, skeletal muscle, regeneration

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EXPLORING HOX GENE FUNCTION ON THE MAINTENANCE OF SKELETAL STEM CELLS

Leclerc, Kevin - *Orthopedic Surgery, NYU Langone, New York, NY, USA*
Remark, Lindsey - *School of Medicine, NYU Langone Health, New York, NY, USA*
Ramsukh, Malissa - *Orthopedic Surgery, NYU Langone Health, New York, NY, USA*
Parente, Paulo - *Orthopedic Surgery, NYU Langone Health, New York, NY, USA*
Palma, Laura - *Orthopedic Surgery, NYU Langone Health, New York, NY, USA*
Morgani, Sophie - *Orthopedic Surgery, NYU Langone Health, New York, NY, USA*
Leucht, Philipp - *Orthopedic Surgery, NYU Langone Health, New York, NY, USA*

Abstract: Adjusting the potency of adult skeletal stem cells (SSCs) will be critical to the successful application of newly derived bone repair therapies. Following their role in patterning the embryonic skeleton, Hox genes remain regionally expressed in SSCs of the adult skeleton. Here, we aim to understand the role Hox genes play in the SSC populations of the tibia. Our results demonstrate that Hox maintains these skeletal stem cells in a multipotential, uncommitted state, thereby inhibiting their differentiation into bone and cartilage. To explore this, we manipulated Hox expression in vitro in tibial periosteal cells, a tissue highly enriched in SSCs and that normally express posterior Hox genes (Hox9-13). After decreasing the expression of Hox in these cells using RNA interference, the “stemness” profile of these cells diminished, taking on more differentiated characteristics – as assayed by cell cycle progression, stem cell markers present on their cell surface, and gene expression of stem cell markers and upregulation of genes associated with tri-lineage differentiation. Similarly, the in vivo deletion of the HoxA cluster in the skeletal stem and progenitor cell domain during tibial injury exhibited a large reduction in stem cell number, highlighting an important stem cell maintenance role for Hox in a regenerative context. Conversely, the overexpression of Hoxa10 in SSCs, via a lentiviral expression vector, demonstrated an increase in the potency of the stem cell pool, as measured by increased stem cell marker expression, a decrease in differentiation and associated markers. Remarkably, Hox overexpression was able to reprogram cells from a more committed progenitor cell state towards a stem cell fate, thereby increasing in vivo self-renewal capacity of the reprogrammed cells. We finally established that this reprogramming ability of Hoxa10 is not universal and is specific to the tibia and pelvis. Instead, the ectopic expression of other Hox genes, namely Hoxa5, Hob8, and Hoxa11, were able to reprogram progenitors deriving from other anatomical regions (the anterior ribs 1-4, spinal vertebrae 5-8, and the radius/ula, respectively) towards stem cells state, demonstrating

the continued functional relevance of the embryonic Hox profile in adult stem cells.

Keywords: Hox, skeletal, reprogramming

TOPIC: NEURAL

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TRANSPLANTED HUMAN STEM CELL-DERIVED INTERNEURONS FUNCTIONALLY INTEGRATE TO PROMOTE RECOVERY AFTER SPINAL CORD INJURY

Zholudeva, Lyandysha V. - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*
Lane, Michael - *Neurobiology and Anatomy, Drexel Univ College of Medicine, Philadelphia, PA, USA*
McDevitt, Todd - *Sana Therapeutics, South San Francisco, CA, USA*
Srivastava, Deepak - *Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, USA*

Abstract: Advances in cell-based strategies offer new promise for some of the most devastating neural injuries like spinal cord injury (SCI). However, to harness the full therapeutic potential of stem cells, it will be necessary to understand how to direct their differentiation to appropriate cell phenotypes and ensure that their phenotype and function persist after transplantation into a pathologic environment. Using pre-clinical SCI in adult rats as a testbed, we tested the therapeutic potential of transplanted human induced pluripotent stem cell (hiPSC)-derived spinal interneurons (SpINs). Building on prior work, we have hypothesized that donor spinal interneurons, known to contribute to plasticity post-injury, will promote novel neuronal relay formation and improve functional outcome. Quantitative PCR, immunocytochemistry, multielectrode array (MEA) analysis and single cell and nuclei RNA sequencing were used to characterize engineered human spinal interneurons prior to transplantation to confirm identity and neuronal function. Neuroanatomical tracing and immunohistochemistry were used to assess transplant integration and connectivity with injured host tissue. Optogenetic activation of hiPSC-SpINs was used to assess development of synaptic connectivity to injured host circuits with time (1- and 2-months post-transplantation). Bilateral terminal diaphragm electromyography was used to quantitatively assess functional contribution of transplanted human SpINs to the recovery of phrenic function months after injury and transplantation. These studies demonstrated that transplanted human SpINs survived and integrated with injured cervical spinal cord circuits, displayed anatomical (e.g., positive for transsynaptic tracing) and functional (e.g., modulate respiratory activity when activated) evidence of connectivity. Having rigorously established improvement in diaphragm muscle activity with objective metrics, this strategy holds great promise to establish motor recovery post-SCI.

Funding Source: This work was supported by the National Institutes of Health, NINDS F32 NS119348, R01 NS104291, the Lisa Dean Moseley Foundation, the Roddenberry Foundation.
Keywords: spinal interneurons, spinal cord injury, phrenic motor network

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PCM1 MODULATES THE POLARIZED ENDOSOME TRAFFICKING BY INTERACTING WITH PAR-3 AND DLIC IN THE ASYMMETRIC DIVISION OF RADIAL GLIA PROGENITORS

Zhao, Xiang - *Zebrafish Cell Atlas, Chan Zuckerberg Biohub, San Francisco, CA, USA*
Royer, Loic - *Cell Atlas, Chan Zuckerberg Biohub, San Francisco, CA, USA*

Abstract: Pericentriolar material 1 (PCM1) was the first satellite protein identified as the master satellite assembly scaffold required for the localization of a number of other satellite components with the pair of centrioles in the centrosome. PCM1 may bind with Dynein complex to generate a pulling force for the cargo movement. Furthermore, the centrosome serves as the primary microtubule-organizing center (MTOC) in animal cells, and recent studies found that the initial difference between the mother versus daughter centrosome in mitotic radial glia cells might trigger the cell fate asymmetry, and the mother centrosome is always inherited by proliferating daughter in Asymmetric cell division (ACD) of mouse RG cells. ACD is a fundamental process of neurogenesis crucial for balancing self-renewal and differentiation in the developing brain. Our recent work has characterized asymmetric pattern of PAR-3 polarity protein segregation along the central spindle via binding through dynein complex together with the endosomal cargos in the ACD of zebrafish radial glia (RG) progenitors. For further exploration of the molecular mechanism regulating such directional cell fate determinant transportation via microtubule binding cargos, we have investigated PCM1 function in the ACD of RG cells by utilizing both pcm1 knockout and knockdown zebrafish embryos. From both live imaging results and Expansion Micrscropy data, we have found that PCM1 can bind cytoplasmic PAR-3 together with Dynein/Dynactin complex to regulate the directional endosomal trafficking in vivo. The dynamic endosome trafficking controlled by PCM1 determines the different daughter cell fate of RG progenitors in ACD. Depletion of PCM1 has caused severe neurodevelopmental disorders by disrupting neurogenesis in developing central nervous system. Our work has offered new insight into the neuropathology of PCM1 in brain disorders and neural degeneration diseases.

Funding Source: Mary Anne Koda-Kimble Seed Award for Innovation in UCSF
Keywords: asymmetric cell division, Par-3 polarity, PCM1

TOPIC: PANCREAS

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MODELING PANCREATIC CANCER WITH PATIENT-DERIVED ORGANOIDS INTEGRATING CANCER-ASSOCIATED FIBROBLASTS

Go, Yoon-Ha - *Microbiology, CHA University School of Medicine, Seongnam*
Choi, Woo Hee - *Microbiology, CHA University School of Medicine, Seongnam, Korea*
Bae, Won Jung - *Pathology, Ajou University School of Medicine, Suwon, Korea*
Jung, Sook-In - *Microbiology, CHA University School of Medicine, Seongnam, Korea*
Cho, Chang-Hoon - *Research & Development Institute,*

ORGANOIDSCIENCES LTD, Seongnam, Korea
Lee, Seung Ah - *Surgery, CHA Bundang Medical Center, Seongnam, Korea*
Park, Joon Seong - *Surgery, Yonsei University College of Medicine, Seoul, Korea*
Ahn, Ji Mi - *Pathology, Ajou University School of Medicine, Suwon, Korea*
Kim, Sung Won - *Otolaryngology-Head and Neck Surgery, The Catholic University of Korea, Seoul, Korea*
Lee, Dakeun - *Pathology, Ajou University School of Medicine, Suwon, Korea*
Yoo, Jongman - *Microbiology, CHA University School of Medicine, Seongnam, Korea*

Abstract: Pancreatic cancer is a devastating disease and is highly resistant to anti-cancer drugs because of its complex microenvironment. Cancer-associated fibroblasts (CAFs) are an important source of extracellular matrix (ECM) components, which alter the physical and chemical properties of pancreatic tissue, thus impairing effective intratumoral drug delivery and resulting in resistance to conventional chemotherapy. The objective of this study was to develop a new cancer organoid model including a fibrous tumor microenvironment (TME) using CAFs. The CAF-integrated pancreatic cancer organoid (CIPCO) model developed in this study histologically mimicked human pancreatic cancer and included ECM production by CAFs. The cancer cell-CAF interaction in the CIPCO promoted epithelial-mesenchymal transition of cancer cells, which was reversed by CAF inhibition using all-trans retinoic acid. Deposition of newly synthesized collagen I in the CIPCO disturbed the delivery of gemcitabine to cancer cells, and treatment with collagenase in-creased the cytotoxic effect of gemcitabine. This model may lead to the development of next-generation cancer organoid models recapitulating the fibrous TME.

Keywords: Pancreatic cancer, Organoid, Tumor microenvironment, Cancer-associated fibroblast, Extracellular matrix, PCO, pancreatic cancer organoid, CIPCO, CAF-integrated pancreatic cancer organoid, EMT, epithelial-mesenchymal transition, CAF, ECM, TME

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

636

EFFICIENT HEPATIC DIFFERENTIATION OF AMNIOTIC MEMBRANE-DERIVED MESENCHYMAL STEM CELLS WITH XENO-FREE CONDITIONS

Choi, Jiwan – *Embryo and Stem Cell Research Center, CHA Advanced Research Institute, Seongnam, Korea*
Kang, Eunju - *Biomedical Science and Center for Embryo and Stem Cell Research, CHA University and CHA Advanced Research Institute, Seongnam, Korea*
Han, Jongsuk - *Biomedical Science, CHA University, Seongnam, Korea*
Kim, Bitnara - *Biomedical Science, CHA University, Seongnam, Korea*
Lee, Yeonmi - *Embryo and Stem Cell Research, CHA Advanced Research Institute, Seongnam, Korea*
So, Seongjun - *Biomedical Science, CHA University, Seongnam, Korea*

Abstract: The liver plays a critical role in the human body, such as detoxification, synthesis, and metabolism. Unfortunately, many people suffer from liver diseases, and the number was increased every year. Recently, stem cell technologies were advanced, and stem cells are expected to replace liver transplantation in the future. Particularly, mesenchymal stem cells (MSCs) are in the spotlight for cell therapy. However, there are obstacles to their use due to low efficiency and xeno-contamination for hepatic differentiation. Thus, we established an efficient protocol for differentiating MSCs into hepatocyte-like cells (HLCs) by analyzing transcriptome-sequencing data. We first analyzed gene and protein expressions in the HLCs differentiated from MSCs of various sources such as umbilical cord matrix (UCM)-MSCs, bone marrow (BM)-MSCs, liver-derived (LD)-MSCs, and amniotic membrane (AM)-MSCs. The AM-MSCs were suitable for stem cell therapy because of their cell size and potential of massive production, but, did not efficiently differentiate into HLCs compared to three other MSC lines. To overcome this matter, we conducted the transcriptome analysis of AM-MSCs and compared them with UCM-MSCs. In AM-MSCs, hepatic development-associated genes, including GATA6, EGF, AFP, and FGF2 were downregulated, and GSK3 was upregulated. The high levels of GSK3 could repress the induction of hepatocytes. Based on these results, we modified an efficient hepatic differentiation protocol using the GSK3 inhibitor, CHIR99021, with various supplements. Moreover, to generate the xeno-free conditioned differentiation protocol, we replaced fetal bovine serum (FBS) with polyvinyl alcohol (PVA). As a result, PVA was improved differentiation ability, such as upregulation of hepatic progenitor, hepatocyte-related, and metabolism markers. Finally, we measured the hepatocyte functions with the genes and protein expressions, secretion of albumin, and activity of CYP3A4. The differentiated HLCs not only synthesized and secreted albumin but also metabolized drugs by the CYP3A4 enzyme.

Funding Source: The present study was supported by research funds from the National Research Foundation of Korea (NRF-2020M3A9E4036527).
Keywords: Mesenchymal stem cells, Amniotic membrane, Hepatic differentiation

TOPIC: PLURIPOTENT STEM CELLS

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TPX2 INDUCES MITOTIC SURVIVAL BY BCL2L1 INDUCTION TRHOUGH YAP1 PROTEIN STABILIZATION IN HUMAN EMBRYONIC STEM CELLS

Kim, Yun-Jeong - *Department of Pharmacy, Seoul National University, Seoul, Korea*
Cha, Hyuk-Jin - *Department of Pharmacy, Seoul National University, Seoul, Korea*

Abstract: For decades, there is emerging evidence that genetic alterations occur in most human embryonic stem cells (hESCs). 'Survival advantage', a typical trait acquired during long-term in vitro culture results from induction of BCL2L1 upon frequent copy number variation (CNV) at 20q11.21, is one of the strongest candidates which lead to genetic alteration by escape from the mitotic stress. However, the underlying mechanism for BCL2L1 induction remains undefined. Of note, abnormal mitosis and 'survival advantage' frequently occurring in the late passage are associated with TPX2 and BCL2L1 expression respectively, located at 20q11.21 loci. In this study, we observed that 20q11.21 CNV was not sufficient for BCL2L1 induction and consequent survival trait in pairs of hESCs and human induced pluripotent stem cells (iPSCs) with normal and 20q11.21 CNV. Inducible expression of TPX2 and bas-

al TPX2 expression due to leakage of inducible system, in hESCs with normal copy number was sufficient to promote BCL2L1 expression and endowed high tolerance to mitotic stress. High Aurora A kinase activity by TPX2 stabilized YAP1 protein to promote YAP1 dependent BCL2L1 expression. Thus, a chemical inhibitor of Aurora A kinase and knockdown of YAP/TAZ significantly abrogated the high tolerance to mitotic stress through suppression of BCL2L1. These results suggest that collective expression of TPX2 and BCL2L1 from CNV at 20q11.21 loci and consequent increase of YAP1 signaling would contribute to augment of genome instability during long-term in vitro culture of hESCs.

Funding Source: National Research Foundation of Korea (NRF) (grant number 2020M3A9E4037905, 2020R1A2C2005914).
Keywords: TPX2, YAP1, BCL2L1

TOPIC: EARLY EMBRYO

962

HOW AN BIOELECTRIC SIGNAL IS INTEGRATED INTO SCALING THE VERTEBRATE DEVELOPMENTAL FIN/LIMB PROGRAM

Antos, Christopher L. - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Yi, Chao - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Wang, Sen - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Zhao, Kun - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Jiang, Xiaowen - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Al-Far, Ezz Al-Din - *Pharmacology and Toxicology, Technische Universitaet Dresden, Dresden, Germany*
Spitters, Tim - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*
Wagner, Michael - *Pharmacology and Toxicology, Technische Universitaet Dresden, Dresden, Germany*
Yan, Xin - *School of Life Sciences and Technology, ShanghaiTech University, Shanghai, China*

Abstract: Generation and regeneration of all organs require scaling mechanisms for the proportional contribution of stem and progenitor cells. In this regard, all anatomical structures form to specific dimensions relative to the body. While the proportional growth control of stem cells is a fundamental phenomenon, it is poorly understood. Previous findings show that the increased activity of the two-pore K+-leak channel Kcnk5b causes allometric growth of zebrafish appendages: however, it remains unknown how the bioelectric activity of this channel is integrated into the scaling mechanisms of these anatomical structures. We found that Kcnk5b is sufficient to concurrently activate development programs in adult, larva and embryo. In vivo mosaic analyses indicate that the Kcnk5-induced activation of developmental gene expression is partially cell autonomous. Our findings also indicate how changes in electrophysiological properties of cells are integrated into the developmental program of limbs: we found that retinoic acid, a morphogen that provides positional information to embryonic cells, transcriptionally induces rcan2, an endogenous protein inhibitor of the phosphatase calcineurin. Calcineurin subsequently dephosphorylates Kcnk5b at serine 345 in the cytoplasmic C-terminus of Kcnk5b. The transgenic overexpression of rcan2 or the inhibition of calcineurin produces the same allometric growth phenotype of Kcnk5b in appendages. Furthermore, treatment with retinoic acid results in the decrease in intracellular K+ similarly to the function of the Kcnk5b K+-leak channel. Subsequently, activation of Kcnk5b will lead to the cell autonomous release of Ca2+ from the endoplasmic reticulum. This release of Ca2+ is required for the expression of at least part of the developmental program. Thus, we provide a molecular cascade for how an electrophysiological mechanism is regulated and how it coordinately controls the activity of different developmental cascades to scale the proportions of an entire anatomical structure: the vertebrate appendage.

Funding Source: National Science Foundation of China ShanghaiTech University Deutsche Forschungsgemeinshaft
Keywords: Tissue scaling, development/regeneration, fin/limbs

TOPIC: ENDOTHELIAL CELLS AND HEMANGIOBLASTS

964

MECHANICAL CONDITIONING REJUVENATES MESENCHYMAL STEM CELLS FROM AGED PATIENTS

Baker, Aaron - *Biomedical Engineering, University of Texas at Austin, TX, USA*
Massidda, Miles - *Biomedical Engineering, University of Texas at Austin, TX, USA*
Demkov, Andrei - *Biomedical Engineering, University of Texas at Austin, TX, USA*
Sices, Aidan - *Biomedical Engineering, University of Texas at Austin, TX, USA*
Lee, Jason - *Biomedical Engineering, University of Texas at Austin, TX, USA*
Lee, Muyoung - *Molecular Biosciences, University of Texas at Austin, TX, USA*
Kim, Jonghwan - *Molecular Biosciences, University of Texas at Austin, TX, USA*

Abstract: Mesenchymal stem cells (MSC) are an appealing therapeutic cell type for many diseases. However, patients with poor health or advanced age often have MSCs with poor regenerative properties. A major limiter of MSC therapies is cellular senescence, which can be caused by poor donor health or by expansion in cell culture. In this work, we examined the hypothesis that mechanical forces combined with small molecule treatment can reduce senescence in MSCs from aged patients and enhance their regenerative capacity. We obtained MSCs from four aged donors (68-92 years old) and conditioned them with physiological mechanical loading and/or an EGFR/ErbB-2/4 inhibitor using a custom mechanical loading system developed in our laboratory. Mechanical loading alone increased proliferation of the MSCs by approximately 50-100% in comparison to non-treated cells. In addition, we found that mechanical loading increased phosphorylation of Akt by five-fold and Cyclin-D1 by six-fold. Following loading, we observed a durable effect on proliferation, with control cells reaching cell cycle arrest after ~5 cumulative doublings whereas mechanically conditioned cells continued to grow up to at least ~9 cumulative doublings. Mechanical load also increased the expression of SIRT1, SIRT6, and SIRT7. In addition, phosphorylation of SIRT1, FoxO1, and FoxO4 was increased in the mechanically loaded group in comparison to control cells. The expression of the DNA damage repair protein Ku80 and antioxidant protein SOD1 were both increased by mechanical

loading and EGFR/ErbB-2/4 inhibitor treatment. A post hoc RNA-seq analysis of MSCs from younger donors using gene set enrichment analysis (GSEA) revealed that mechanical conditioned cells had reduced expression of gene sets associated with high passage MSCs, genes upregulated in aged donor derived MSCs, and genes upregulated in senescent cells. Taken together, our findings suggest that mechanical forces can be used to improve proliferation and reduce senescence in MSCs and may provide a practical method to enhance the regenerative function of these cells in aged patients.

Funding Source: American Heart Association (grant #17IRG33410888), the DOD CDMRP (grant #W81XWH-16-1-0580 & W81XWH-16-1-0582) and National Institutes of Health (grant #1R21EB023551-01, 1R21EB024147-01A1 & 1R01HL141761-01) to Aaron B. Baker.
Keywords: Mesenchymal stem cell therapy, Cellular senescence, Biomechanical conditioning

TOPIC: EPITHELIAL_SKIN

966

CELLULAR AND EPIGENETIC DYNAMICS BY WHICH MEDIATOR 1 ABLATION CHANGES CELL FATE OF DENTAL EPITHELIA TO THAT GENERATING HAIR

Oda, Yuko - *Medicine, University of California and VA Medical Center, San Francisco, CA, USA*
Yoshizaki, Keigo - *Dental Science, Kyushu University, Fukuoka, Japan*
Thaler, Roman - *Orthopedic research, Mayo Clinic, Rochester, NY, USA*
Fukumoto, Satoshi - *Dental Science, Kyushu University and Tohoku University, Fukuoka and Sendai, Japan*
Bikle, Daniel - *Medicine, University of California San Francisco and VA Medical Center San Francisco, CA, USA*

Abstract: Ectodermal lineages underlying tooth enamel and hair development are tightly controlled by transcriptional and epigenetic programs. However, genetic removal of one epigenetic factor, Mediator 1 (Med1), changes their cell fate resulting in the appearance of hair on incisor and defective enamel regeneration in mice. Here, we evaluated the cellular and epigenetic dynamics by which Med1 deficient dental epithelial stem cells are driven into an epidermal lineage. In contrast to natural hair development in the skin, Med1 null incisors generate hair through unique cellular processes without the presence of any hair follicles. Nevertheless, Med1 deficient dental stem cells switch to a developmental program which features the transcription toward not only hair but also interfollicular epidermis that are sequentially induced in the skin. In fact, cultured Med1 null dental epithelial cells follow a p63/53 driven epidermal transcriptional program, reminiscent to skin. A comprehensive transcriptional and epigenetic comparison reveal that Med1 deficiency induces the expression of epidermal fate transcription factors (TFs) such as Cebpb and Fosl2 but represses enamel fate TFs such as Pitx2 in dental epithelia. These changes are accompanied by modifications in chromatin accessibility at the promoter regions of these genes but no substantial changes are observed at the super-enhancer (SE) level, implicating that Med1 is critical to link SEs to promoters to control transcription and presence of Med1 lacking alternative epigenetic machinery. Our results demonstrate that Med1 deficiency causes a lineage switch in mouse incisors by using unique cellular process and epigenetic machinery, that may be developmentally conserved as epigenetic memory in ectodermal epithelia. Our studies point to cellular and epigenetic mechanisms supporting the plasticity of tissue stem cells, providing new insights into the ectodermal regeneration.

Funding Source: NIH grant R21 DE025357 (YO), R01 AR050023 (DDB), VA Merit I01 BX003814-01 (DDB), and Japanese grants-in-Aid for Scientific Research 18H03012, 17K19765 (KY).
Keywords: Cell fate, tissue stem cells, epigenetic

TOPIC: HEMATOPOIETIC SYSTEM

968

DEVELOPMENT OF EX VIVO HEMATOPOIETIC STEM CELL ASSAYS USING A HIGHLY SELECTIVE EXPANSION SYSTEM

Igarashi, Kyomi J. - *Genetics, Stanford University, Stanford, CA, USA*
Hsu, Ian - *MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK*
Khoo, Hwei Minn - *MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK*
Nakauchi, Hiromitsu - *Genetics, Stanford University, Stanford, CA, USA*
Wilkinson, Adam - *MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK*

Abstract: While the self-renewing and multipotent hematopoietic stem cells (HSCs) can maintain and reconstitute the whole blood and immune system, their scarcity in vivo has hindered its use in blood disease treatment and in vitro characterization studies. Despite developments in ex vivo expansion methods, achieving high HSC selectivity in these cultures remains difficult. We have previously introduced a polyvinyl alcohol (PVA)-based HSC culture system that can expand mouse bone marrow HSCs 236- to 899-fold over the span of a month. However, HSCs comprise less than 10% of the total culture, with the majority being differentiated progenitors and mature hematopoietic cells. We have now significantly increased the HSC selectivity of this system by decreasing oxygen (O2) culture concentrations from the standard 20% to hypoxic 5%. The selectivity in 5% O2 conditions is demonstrated by: (1) an almost complete depletion of differentiated progenitors and mature hematopoietic cells in 4-week HSC-derived cultures and (2) selective expansion of HSCs from heterogeneous populations (unfractionated bone marrow and fetal hematopoietic tissues), eliminating the need for initial immunophenotyping, a necessary step in 20% O2 cultures. The system recapitulates the in vivo HSC expansion potential throughout the stages of definitive developmental hematopoiesis, with ex vivo expansion observed only in tissues and at embryonic time points when definitive HSCs are known to be present in vivo. This culture condition also allows expandable HSC frequencies to be estimated from heterogeneous cell populations using an ex vivo limiting dilution assay approach. To investigate the mechanism of this selectivity, we performed various transcriptomic analyses. This revealed an upregulation of stress response pathway factors (e.g., Atf5) in 20% O2, which has been reported to promote HSC lineage commitment. In contrast, an upregulation of sterol and cholesterol metabolism pathways was observed in the 5% O2 cultures. Enrichment of hypoxia inducible factor 1 (HIF1) activity was also observed in hypoxic conditions. In summary, this highly selective culture system can be used to develop powerful new ex vivo assays for HSC activity,

while also providing potential time- and cost-efficient alternatives to in vivo limiting dilution assays.

Funding Source: b
Keywords: Hematopoietic stem cell, Ex vivo expansion, Oxygen/hypoxia

TOPIC: MUSCULOSKELETAL

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UNIVERSAL ALLOGENIC AND FAILSAFE™ NUCLEUS PULPOSUS PROGENITOR CELLS FOR INTERVERTEBRAL DISC REGENERATION

Zhu, Mengxia - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, China*
Tam, Vivian - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, China*
Warin, Julie - *INSERM UMR 1229, Regenerative Medicine and Skeleton, Nantes Université, Nantes, France*
Nagy, Andras - *Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; Australian Regenerative Medicine Institute, Monash University, Melbourne, VIC, Australia; panCELLA, Inc., Toronto, ON, Canada*
Camus, Anne – *INSERM UMR 1229, Regenerative Medicine and Skeleton, Nantes Université, Nantes, France*
Chan, Danny - *School of Biomedical Sciences, The University of Hong Kong, Hong Kong SAR, China*

Abstract: Intervertebral disc degeneration (IDD) causes back pain affecting quality of life of which there is no effective biological treatments. The central nucleus pulposus (NP) and its cellular content is key to a healthy IVD. Notochordal-like cells (NLCs) serve as progenitors that give rise to NP cells and are determined by markers including TBXT and KRT8, identified from animal and human studies. A decline in NLCs and differentiation of NP cells to fibrotic cells coincides with the onset and severity of IDD. A potential cell-based therapy for IDD consists of NLCs derived from pluripotent stem cells. Here, we use a genetically engineered "immuno-cloaked and FAILSAFETM" system, where these cells can evade host immunity, and tumorigenic cells can be eliminated. These cells were targeted with fluorescent reporters marking NLC proteins (TBXT and TAGLN) and differentiated towards NLCs. For in vitro differentiation of these cells towards NLCs, we tested two existing protocols for optimization. Cells were cultured on plates coated with Laminin-511 or Geltrex Matrix at the onset to maintain pluripotency. Cells were then induced to differentiate along the mesoderm lineage followed by differentiation towards NLCs. Activin A was used for mesoderm differentiation over a period of 2 days that showed high efficiency with the expected reduction in pluripotency marker (POU5F1), and increased expression of mesoderm markers (NOTO, FOXA2) during this differentiation phase. For induction towards NLCs, we tested different combinations of Activin A (inducer of ACTIVIN/NODAL), FGF2 (inducer of XNOT), Noggin (inhibitor of BMP), CHIR99021 (inducer of WNT) and AGN193109 (inhibitor of retinoic acid). The optimal combination of FGF2, Noggin, CHIR99021 and AGN193109 induced significant increase in the expression of key NLC markers such as TBXT, KRT8 and HOPX when the cells were seeded as single cells and cultured in monolayer. Seeding of cells as spheroids in monolayer culture showed little improvement. Differentiation of cells towards NLCs can provide a source of committed progenitor cells for disc regeneration. These targeted immuno-cloaked and FAILSAFE™ NLCs will be tested for treating IDD in animal models in future.

Funding Source: The RGC European Union - Hong Kong Research and Innovation Cooperation Co-funding Mechanism (iPSpine) (E-HKU703/18)
Keywords: Intervertebral Disc Degeneration, Notochordal-like Cells, Cell Therapy

TOPIC: NEURAL

972

LONG-TREM FUNCTIONAL REGENERATION OF RADIATION-DAMAGED SALIVARY GLANDS THROUGH LOCAL DELIVERY OF NEUROGENIC HYDROGEL

Li, Jianlong - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Sudiwala, Sonia - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Berthoin, Lionel - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
May, Alison - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Mohabbat, Seayar - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Gaylord, Eliza - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Cruz Pacheco, Noel - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*
Alsberg, Eben - *Bioengineering and Orthopaedics, University of Illinois, Chicago, IL, USA*
Bahney, Chelsea - *4Steadman Philippon Research Institute. Center for Regenerative and Personalized Medicine, University of California, San Francisco, CA, USA*
Knox, Sarah - *Cell and Tissue Biology, University of California, San Francisco, CA, USA*

Abstract: Salivary gland acini are severely depleted in response to radiotherapy for head and neck cancer, leading to loss of saliva and extensive oro-digestive complications. With no regenerative therapies available, organ dysfunction is irreversible. Here, we demonstrate that long-term functional regeneration of irradiated salivary glands can be achieved via sustained delivery of the muscarinic receptor agonist, cevimeline. Using the murine system, we show acinar cell replacement occurs during the first week following radiation treatment but is lost by the second week, with extensive acinar cell degeneration, dysfunction and denervation occurring within 2-3 months. However, delivery of a single localized dose of cevimeline encapsulated in an alginate hydrogel at 2 weeks post-radiation was sufficient not only to repopulate and preserve innervated salivary acinar cells but to also retain physiological saliva secretion at non-irradiated levels for up to 8-weeks. Strikingly, combining the single dose of cevimeline hydrogel with weekly injections of free cevimeline preserved physiological saliva secretion and prevented the degeneration and denervation of glandular architecture over the 12-week time period tested. Thus, we reveal radiation-damaged salivary glands are functionally regenerated through local application of a neurogenic hydrogel, an outcome that has major implications for the restoration of human salivary glands.

Funding Source: TRDRP under award #28IR-0071 (SMK); NIDCR of the National Institutes of Health (NIH) under award numbers

#R35DE028255 (SMK); C-DOCTOR, Technology Development Award #U24DE026914 (SMK, CSB, EA).
Keywords: tissue regeneration, radiation damage, muscarinic receptor agonist

TOPIC: PLACENTA AND UMBILICAL CORD DERIVED CELLS

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THE DISTRIBUTION AND NEUROPROTECTIVE EFFECT OF WJ-MSCS TRANSPLANTED IN 3D HYDROGELS IN THE EXPERIMENTAL MODEL OF STROKE

Lech, Wioletta - *Department of Stem Cell Bioengineering, IMDiK PAN, Warszawa, Poland*
Kot, Marta - *Department of Stem Cell Bioengineering, IMDiK PAN, Warsaw, Poland*
Zychowicz, Marzena - *Department of Stem Cell Bioengineering, IMDiK PAN, Warsaw, Poland*
Buzanska, Leonora - *Department of Stem Cell Bioengineering, IMDiK PAN, Warsaw, Poland*

Abstract: Stroke is one of the most common causes of death in humans in highly developed countries. Thus in stroke-related regenerative medicine applications precise protocols for therapeutically competent stem cells are urgently needed. To protect stem cells from strong recipient immune response after transplantation while enhancing their regenerative properties, we established procedures that include preconditioning in biomimetic microenvironment as physiological normoxia and/or encapsulation in hydrogels. The main goal of this study was the evaluation of the host nervous tissue response to the transplantation of WJ-MSCs in the experimental model of brain injury: transplant distribution, area of lesion site and the expression of neurotrophins. WJ-MSCs were cultured in different oxygen conditions (21%O2 or 5%O2) and then transplanted into injured rat brains in saline or in hydrogel scaffolds made from human platelet lysate (3D PL). The distribution of SPIO-Molday ION rhodamine-labeled WJ-MSCs transplanted in vivo was analyzed by MRI in control and experimental conditions. To evaluate the recipient's response striatum from the injured area and cerebrospinal fluid (CSF) were collected at different time points (1, 7, 14, 21 days post-transplantation). After the injury, a significant damage of brain tissue was observed. The signal generated by labelled cells transplanted in saline and in 3D PL was detected in the lesion area throughout the 21-days of experiment. A significant reduction in the lesion size was observed, especially after transplantation of cells encapsulated in the scaffolds. Moreover, the increased mRNA expression of rat BDNF and GDNF has been observed, with the maximum level noticed 21 days after transplantation of 3D PL WJ-MSCs preconditioned in 5% O2. The concentration of analyzed neurotrophins in rat CSF significantly increased after transplantation, especially in PL group compared to WJ-MSCs injected in saline. Hydrogels composed of human plasma-derived proteins are a promising carriers for cell transplantation and can promote neuroprotection as well as stimulate recipient tissue for regeneration. The obtained results can be used to establish appropriate protocols for preconditioning and transplantation of stem cells for the treatment of central nervous system disorders.

Funding Source: This work was supported by NSC grant No. 2019/35/N/NZ5/03723 and statutory funds to MMRI PAS.
Keywords: stroke, Wharton's jelly mesenchymal stem/stromal cells, hydrogel scaffolds