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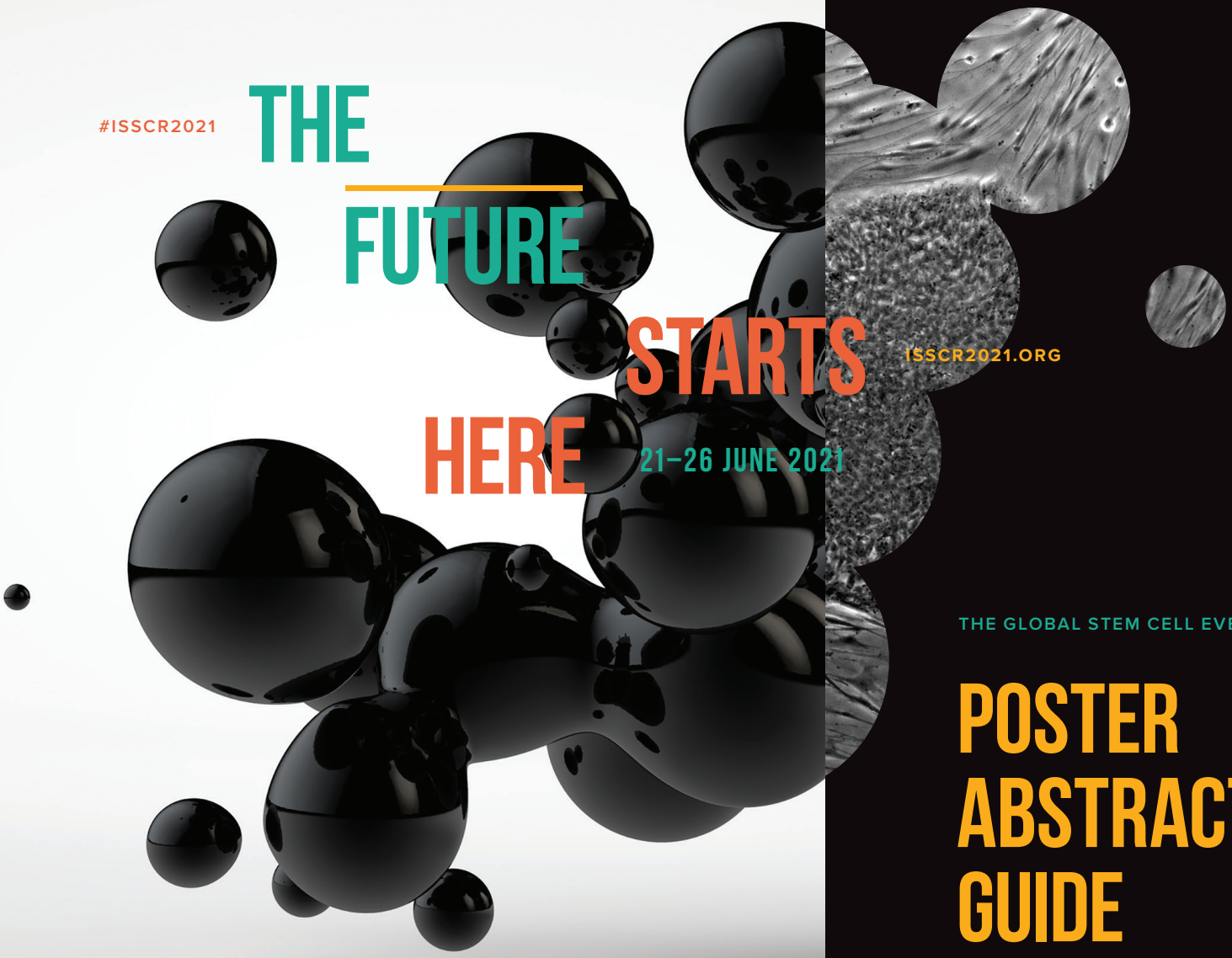

THE GLOBAL STEM CELL EVENT

POSTER ABSTRACT GUIDE

ISSCR

POSTER SESSIONS

All times in EDT

TUESDAY, JUNE 22

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WEDNESDAY, JUNE 23

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12:00 - 13:00 EDT

POSTER SESSION 1

NEW TECHNOLOGIES

Poster: 101

STATISTICAL MODELING TO RANK HUMAN MESENCHYMAL STROMAL CELL ENHANCEMENT METHODS AND EVALUATE DONOR HETEROGENEITY

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Mesenchymal stromal cells (MSCs) are widely investigated for their anti-inflammatory and angiogenic properties; however, insufficient potency and donor heterogeneity can limit MSC treatment efficacy. The Viswanathan lab has pioneered a xeno-free 3D aggregate culture method to enhance MSC immunomodulatory functions. Notably, hypoxic culture also augments MSC paracrine functions. Our objective is to characterize the immunomodulatory and angiogenic properties of 3D and hypoxic MSCs, and to apply desirability profiling to statistically rank MSC potency across five human MSC donors. Adipose-derived MSCs (AD-MSCs) were subject to 3D or hypoxic culture for 16 h, using 2D normoxic MSCs as controls. Morphometric analysis revealed that 3D aggregates displayed mean feret diameters of 36.12 µm ± 4.34 µm (mean ± SD) while single cells from dissociated aggregates had significantly smaller diameters than 2D MSCs, characteristic of a more functionally primitive MSC phenotype. Gene expression experiments were performed on AD-MSCs licensed with pro-inflammatory cytokines (IFNγ, TNFα, IL-1β) using a NanoString custom 50-gene panel. Principal component analysis of the gene expression data revealed that 3D AD-MSCs have a distinct signature compared to 2D normoxic and hypoxic AD-MSCs, with significantly differentially expressed genes (DEGs) associated with immunomodulatory (17 DEGs) and angiogenic (12 DEGs) functions. Desirability analysis of the gene expression data demonstrated higher scores for 3D AD MSCs for both immunomodulatory and angiogenic properties, suggesting that 3D AD-MSCs have enhanced immunomodulatory and pro-angiogenic gene expression profiles. Intermediate scores were observed for 2D hypoxic AD-MSCs, with 2D normoxic AD-MSCs receiving the lowest scores. Desirability analysis also revealed an impact of donor heterogeneity on the immunomodulatory and angiogenic gene expression profiles, suggesting that different donors are inherently more immunomodulatory or angiogenic. Overall, this work suggests an advantage of 3D AD-MSCs over conventional 2D culture and highlights the importance of donor selection for MSC applications requiring immunomodulatory or pro-angiogenic therapy. In ongoing studies, the gene expression data will be validated within functional in vitro assays.

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, statistical modelling, 3D culture

Poster: 102

RAPID, NON-ENZYMATIC, HIGH-YIELD ADIPOSE TISSUE STEM CELL PROCESSING

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Synova Life Sciences has developed a method using mechanical energy to rapidly dissociate adipose tissue and increase cell yields while minimizing cellular stress and damage. The output is a high-quality stromal vascular fraction and micro-fragmented adipose tissue. In direct comparisons of this method with a standard enzymatic digestion, total nucleated cell counts of the stromal vascular fraction obtained from this method were typically 1.3 to 2.5 times higher. Processing time for 50 mL of adipose tissue using this method was approximately 3 minutes compared to a 45-minute enzymatic digestion using 0.1% collagenase type I, additional neutralization, and washing. Because the method is purely mechanical, no neutralization step is needed and the cellular fraction can be concentrated and deployed immediately. Microscopic examination at 400x of the monocytes comparing the two processing methods revealed the mechanically processed cells to be smaller, highly circular, and less prone to clumping. The enzymatically processed cells tended to be larger, slightly less circular, and had a tendency to clump, which can be indicative of cellular damage and stress. Viabilities ranged approximately between 85% and 90%, and were assessed using calcein AM and DAPI staining. Further plating of the cells for adherent lines showed high levels of attachment from both processes, with higher densities and more rapid confluence in the mechanically processed cells. Analysis using spectral flow cytometry confirmed the identity of adipose-derived mesenchymal stem cell colonies on the plates at approximately 97% for CD90+, CD105+, CD73+ cells. The speed, yield, and simplicity of this mechanical method of adipose tissue processing removes many of the barriers of enzymatic processing, and overcomes the low-yield disadvantages of existing mechanical methods. For research use, tissue engineering, clinical trials, and translation into cell therapies, therapeutics, and point-of-care, this is a very promising method for obtaining autologous cells and for establishing allogeneic cell lines.

Keywords: adipose tissue processing, stromal vascular fraction, adipose derived mesenchymal stem cells

Poster: 103

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

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Development of biologically relevant and predictive cell-based assays for compound screening and toxicity assessment is a major challenge in drug discovery. The focus of this study was to establish high-throughput compatible cardiotoxicity assays

using human induced pluripotent stem cell (iPSC)-derived cardiomyocytes. Using human iPSC-derived cardiomyocytes as an in vitro model, we evaluated the responses and concentration dependence to 28 drugs linked to low, intermediate, and high torsades de pointes (TdP) risk categories. The impact of various compounds on the beating rates and patterns of cardiomyocyte spontaneous activity was monitored by changes in intracellular Ca2+ oscillations measured by fast kinetic fluorescence with calcium-sensitive dyes on the FLIPR Penta system. We describe a method for the complex analysis of calcium oscillations that allows detection and multi-parametric characterization of oscillation peaks and patterns. In addition to detection oscillation rates, peak width and amplitude, the method allows characterization of complex patterns, secondary peaks, waveform irregularities, and more than 20 other important readouts. Compound-induced pro-arrhythmic effects such as Early After-Depolarization (EADs)-like events or peak prolongations can be easily identified and flagged. In addition, cellular and mitochondrial toxicity were assessed in the follow-up assay by high-content imaging. We characterized the concentration-dependent effects of 28 compounds on different readouts and demonstrated that presence of EAD-like events, peak prolongations, and pattern irregularities detectable in the assay at concentrations comparable with concentrations in the appropriate concentration in blood (Cmax), can be used as a strong predictive indicator of cardiac arrhythmia in vivo. The results demonstrate the utility of the method for high-throughput screening and detection of drug-induced proarrhythmic effects in vitro.

Keywords: Cardiiomyocytes, Calcium oscillations, High-content imaging

Poster: 104

BIOMATERIAL MICROSPHERE ENCAPSULATION OF PLURIPOTENT STEM CELLS IMPROVED CONTRACTILITY OF RESULTING CARDIAC TISSUE

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Scaling up production of cardiomyocytes (CMs) for regenerative and drug-testing applications requires a suspension-based culture system. Building on our prior work demonstrating cardiac differentiation of hydrogel-encapsulated human induced pluripotent stem cell (hiPSCs) and CM maturation including T-tubule formation, here we established a microfluidic system to rapidly encapsulate hiPSCs within PEG-fibrinogen (PF) hydrogel microspheres and differentiated these microspheres in suspension culture, which is amenable for scale-up. HiPSCs were able to completely remodel and degrade the PF, consistently forming spheroidal function cardiac tissues. To investigate the effect of encapsulation on differentiation and resulting cardiac tissue function, we compared tissues formed using our microsphere-supported differentiation approach to self-aggregated embryoid body (EB) differentiation. In comparison to EBs, microspheres were more consistent in size and shape both initially and over time and within and between batches. Resulting microspheres had an initial diameter of 673±22 µm (coefficient of variance, CV=0.03) and axial ratio of 1.03±0.01 (CV=0.01), while EBs had an initial diameter of 145±34 µm (CV=0.24) and axial ratio of 1.15±0.04 (CV=0.03). Microspheres had a significantly higher number of CMs (2.8 ± 1.8) at day 10 per initial hiPSC than EBs (0.6±0.12). In addition, the microspheres had a higher percentage of CMs on day 10 in comparison to EBs; differentiation of microsphere-encapsulated hiPSCs resulted in 74.73±13.82% CMs while the EB CM yield was 55.98±16.12 % based flow cytometry data (cTnT+, MF20+, n=14 and 5 batches, respectively, $p<0.05$). Myocardial contractility was also enhanced in cardiac microspheres compared to EBs (similar CM content, day 10); maximum contraction velocity was 4x higher for microspheres than EBs (138.85±17.57 µm/s versus 33.4±5.1 µm/s) and maximum relaxation velocity was 14x higher (97±7.2 µm/s versus 7.42±0.44 µm/s). In conclusion, the microsphere-encapsulated hiPSCs differentiated into contracting cardiac tissues, with higher CM content and faster contractility than similarly differentiated EBs, demonstrating the potential of encapsulation to enhance the consistency, yield, and functionality of cardiac tissue produced in scalable culture systems.

Funding Source: NSF grant #1743445 Department of Education GAANN grant #P200A180087

Keywords: Hydrogel, Microsphere, Cardiomyocyte

Poster: 105

SCALABLE EXPANSION CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING MICROCARRIERS

Kurakazu, Tomoaki[1], Sato, Sho[2], Hibino, Ryo[3], Mifune, Maki[4], Oba, Takahiro[3], Kagawa, Hideaki[4], Beardsley, Nathaniel[4], Bakshy, Kiranmayee[4], Dickerson, Sarah[4], Koonce, Chad[4], Fuhrken, Peter[5]

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Human induced pluripotent stem cells (hiPSCs) have unlimited potential for cell therapy products to address unmet medical needs and disease modeling tools to unlock new possibilities for drug discovery. A scalable and reproducible cell expansion process is important for the translation from research to commercial manufacturing. Moreover, for clinical application, it is advantageous to use a single-use, closed and controlled environment. At FUJIFILM Cellular Dynamics, Inc. (FCDI), a leading developer and manufacturer of hiPSC derived cells used in drug discovery, toxicity testing, stem cell banking, and cell therapy development, we have experience in process optimization with various culture formats. Here, we present microcarrier-based hiPSC three-dimensional (3D) culture up to 10L scale. Under optimized conditions with vertical wheel mixing, we achieved up to 10.4 billion cell yield after 9 days while maintaining comparable cell quality including pluripotency marker expression and viability. In addition, closed cell washing and concentration by counterflow centrifugation was demonstrated with high viability and a recovery ratio over 85%. Post-processed iPSCs were successfully tested in multiple downstream applications including plating into 2D, making 3D aggregates for differentiation, and cryobanking. Together, these established iPSC scale-up and processing methods allow for the routine manufacture of > 10 billion iPSCs per batch.

Keywords: Human Induced Pluripotent Stem Cells, Scale up, Microcarriers

Poster: 106

CHARACTERIZATION OF PLAKOGLOBIN KNOCK-OUT IPSCS LINES DURING CARDIAC DIFFERENTIATION

Castañeda, Sheila L.[1], Mobbs, Alan Miqueas[1], Scarafia, Maria Agustina[1], Waisman, Ariel[1], La Greca, Alejandro Damian[1], Luzzani, Carlos Daniel[1], Sevlever, Gustavo[1], Atorrasagasti, Catalina[2], Miriuka, Santiago Gabriel[1], Moro, Lucia Natalia[1]

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Plakoglobin (PKG), also known as γ-catenin, is a member of the armadillo family of proteins and it is an important component of both desmosomes and adherens junctions, playing a vital role in cell-cell adhesion. Similar to β-catenin, PKG can also be found in the nucleus and is capable of participating in cell signaling as the Wnt/β-catenin pathway. PKG knockout mice resulted in lethality, which was attributed to defective desmosome formation and inpaired cardiac development. The aim of this work was to characterize two PKG knockout iPSCs lines generated by CRISPR/Cas9 during cardiomyocyte differentiation (PKG-KO1 and PKG-KO2), determining the relevance of this protein and the phenotype of the cells. To achieve this, we used a cardiomyocyte differentiation protocol in monolayer using regulators of canonical Wnt signaling. For gene expression analysis, RNA samples were taken on day 0, day 3.5, day 14 and day 21 of differentiation, from 3 independent experiments. At the pluripotent state and comparing to wild-type iPSCs, PKG-KO cell lines showed higher expression of Pkp2, lower expression of Connexin 43 and similar expression levels of β-catenin, E-Cadherin, Oct4, Nanog, Cyclin-E1 and desmosomal proteins (DSG-2, DSC-2 and DSP). During differentiation, we observed a significant decrease in the expression of the pluripotency genes (Oct4 and Nanog), an increase of the expression of all desmosomal genes (DSG-2, DSC-2, DSP, PKP2 and PKG) and mesoderm-cardiac genes (β-catenin, Brachyury, Mesp1, TBX6, Connexin-43, SPARC, NKX2.5, MYH7, Islet1 and cTnT) either in WT or in the PKG-KO cell lines. However, the PKG-KO cell lines showed a lower increase of expression of the cardiac genes by day 14 and 21 of the protocol compared to the WT cells. This result could be related to their lower contractility capacity shown by day 10 of cardiac differentiation and lower percentage of cTNT+ cells observed by day 14 (28±1.5% vs. 11.6±7.3% and 3±1.7% for WT, PKG-KO1 and PKG-KO2, respectively). In conclusion, the absence of PKG is determinant to the cardiomyocyte differentiation capacity of the iPSCs. This work is important since PKG mutations have been described in an inherited heart muscle disease known as arrhythmogenic cardiomyopathy (ACM) and little is known about the relevance of this protein during the development of human cardiac tissue.

Keywords: CRISPR/Cas9, Cardiac Differentiation, IPSCS

Poster: 107

QUALITY CONTROL ASSAYS FOR PLURIPOTENT STEM CELL LINE BANKS: KEY OBSERVATIONS FROM 2 YEARS OF TESTING

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Human pluripotent stem cells (hPSCs) have been globally recognized as tools for disease modeling, drug discovery and cell therapy. However, in order to ensure the quality of hPSCs, which is critical during cell line generation, repository deposition and clinical approaches, there was a need to develop standardized quality control methods that can ensure the pluripotency, differentiation potential and genomic stability of hiPSC. We leveraged the application of existing methodologies to build several hPSC characterization offerings at Thermo Fisher Scientific. Assays available at the DNA level include Karyostat™ and the Oncomine™ Comprensive Assay to look at genomic stability and cancer prone mutations respectively. For pluripotency, we can screen hPSC samples using Pluritest™ or the TaqMan™ hPSC Scorecard™ Panel to probe transcripts of unknown samples using a whole genome expression array or focused qPCR panel respectively. The latter assay is also routinely used with embryoid bodies or directed differentiation to understand lineage bias of generated hPSC lines. Here, we present an overview of results using historical submitted sample data generated using Karyostat, Pluritest and Taqman hPSC Scorecard assays to get a sense of the overall quality of hPSC lines used in the field. Presented results indicate trends around the observed incidence and recurrence of chromosomal aberrations, as well as a deeper dive in the pluripotency assessment of hPSC samples.

Keywords: pluripotent stem cells, genomic stability, pluripotency

Poster: 108

ENGINEERING IN VITRO MODEL OF PERFUSABLE ORGAN-SPECIFIC MICROVASCULARTURE FROM HIPSC-DERIVED VASCULAR ORGANOIDS

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Vascular development starts from mesodermal precursors, which differentiate and mature into hierarchical perfusable networks with organ-specific characteristics. Efforts are made towards replicating perfusable vasculature in vitro for investigating vascular remodeling, tissue regeneration, and vascular diseases. However, establishing actively remodeling vasculature is challenged by the lack of controlled cell renewal and perfusion. Recently developed human induced pluripotent stem cell-derived vascular organoid (hVOs) harbors 3D self-assembled endothelial cells (ECs), supportive perivascular cells, and the progenitor cells which provide the relevant cell types for pliable modeling of complex vasculature. A remaining critical challenge is to establish and control perfusion in the hVOs. Here, we aim to address these challenges by combining

hVO technology with microvascular engineering to establish a perfusable organ-specific microvascular niche in vitro and understand flow- and parenchyma-driven EC fate specification, vascular remodeling, and vessel function. We applied a soft-lithography-based micropatterning and injection-molding to fabricate a patterned vascular network in collagen hydrogels. The hVOs were inserted into the center of microvessels surrounded by pre-formed microchannels seeded with human umbilical vein endothelial cells (HUVECs). The preliminary results show that capillary extensions from hVOs sprout towards HUVEC networks. At day 7, dextran perfusion results in intraluminal perfusion of hVO through the adjoining capillaries which suggests the formation of a robust anastomosis between hVOs and HUVEC microchannels. Furthermore, 7-day co-culture of hVOs with renal proximal tubule epithelial cells shows enhanced expression of PLVAP, a key regulator of fenestral diaphragm formation in kidney ECs, indicating that kidney-specific EC phenotype can be induced within hVOs through signaling interactions with adjacent parenchymal cells. Future studies will investigate hierarchical network remodeling in hVOs in presence of intraluminal flow and pressure gradient, and the extent to which hVOs can acquire organotypic phenotype and function.

Funding Source: This work is supported by R01HL141570, UG3TR002158, and 1R61HL154250 awarded to Y.Z and ISCRM fellowship awarded to Y.J.S.

Keywords: Vascular organoid, Stem cell engineering, Tissue engineering

Poster: 109

DEEP LEARNING-BASED IMAGE ANALYSIS FOR LABEL-FREE LIVE MONITORING OF IPSC AND 3D ORGANOID CULTURES

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Complex biological models such as organoids, tumoroids, and spheroids are gaining popularity in many research areas and biomedical applications because they are more representative of the in vivo tissues compared to monolayer 2D cultured cells. Recent advances in organoid culture techniques has led to the ability to grow microtissues such as lung, liver, intestine, and brain derived from human iPSCs (induced pluripotent stem cells) or adult stem cells. These organoids offer huge potential in disease modeling, drug screening, toxicity studies, and host-microbe interactions. A present challenge in using 3D model systems is their limited scalability. Organoids are often generated from patient-derived iPSCs or CRISPR-modified cells. The manual culture of iPSCs introduces issues which includes increased variability of cell quality and potential human errors especially when processing large numbers of samples. As such, automation is increasingly being used to culture more consistent and reproducible iPSC lines and organoids. A major requirement of an automated culture system is the ability to monitor live tissues. Here, we used an automated imaging platform to monitor cells and organoids in culture. We used an artificial intelligence (AI) deep learning tool to carry out automated image analysis of iPSC colonies, organoids, and tumoroids (imaged in brightfield). The software tool provides an intuitive user interface to annotate and train models based on user provided images. In contrast to non-AI based image analysis where users are required to provide analysis settings, the deep learning based module enables automatic detection of the objects of interest with minimal human intervention, e.g. detect stem cell colonies or organoids. Analysis output includes spatial (area, form factor, perimeter, and diameter), intensity, and texture measurements. We demonstrate the feasibility of using AI-based object detection and phenotypic characterization for three complex cell models: expansion of iPSCs, development of 3D lung organoids, and effects of anti-cancer drugs on tumoroids. These results support the integration of deep-learning image analysis methods into an automation workflow that will greatly facilitate large scale generation of high quality iPSCs and organoids for downstream applications.

Keywords: Deep learning, label-free imaging and analysis, iPSC monitoring

Poster: 110

METASTATIC NICHE MODELING USING HUMAN PSC-DERIVED LUNG ORGANOIDS

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Metastasis is the main reason behind cancer-related mortality, with limited therapeutic opportunities currently available. Due to complicated detection and modeling in mice, numerous events early in the metastatic cascade remain yet to be elucidated. Here, we propose to leverage the human pluripotent stem cell- (hPSC) derived lung organoid differentiation to model lung (pre-)metastatic niche conditioning by breast cancer cells. We set up different co-culture conditions, allowing to query both distal and proximal interaction between hPSC-derived lung organoids and breast epithelial cells, differing in their tumorigenic and metastatic potential. Our data suggests that we can recapitulate multiple molecular events associated with breast cancer progression, previously observed in mouse models and patient biopsies. While we continue to investigate the systematic changes in the lung microenvironment induced by the breast cancer cells ex vivo, single cell RNAseq analyses suggest that the presence of breast cancer cells impact the cell fate decisions in the lung organoid lineages. We anticipate our results to give precedent to a faithful ex vivo modeling of early metastatic events using human stem cell-derived organoids.

Keywords: hPSC-derived lung organoids, breast cancer, metastatic niche

Poster: 111

PROGRAMMABLE TRANSCRIPTIONAL REPRESSION WITH A NOVEL DCAS9 FUSION PROTEIN AND SYNTHETIC GUIDE RNAS

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The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated 9 proteins) system derived from Streptococcus pyogenes commonly used for genome editing has also been adapted for transcriptional modulation and epigenetic engineering, such as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi). For CRISPRi, the guide RNA forms a complex with a nuclease-deactivated Cas9 (dCas9, D10A, and H840A), fused to one or more transcriptional effectors. The guide RNAs target the region proximal to and downstream of the transcriptional start site (TSS) to down-regulate the target gene's expression. First-generation CRISPRi systems utilize the Krüppel associated box (KRAB) domain from zinc finger protein 10 (KOX1) fused to dCas9 (dCas9-KRAB) as a transcriptional repressor, an approach shown to be more target-specific than other existing technologies for temporary gene repression. Given that this CRISPR-based approach can also result in less robust repression of the target gene(s), there is a need for identifying ways to improve its efficiency.Here we describe a novel fusion protein for CRISPRi comprised of domains from two human transcriptional repressors, Sal-like protein 1 (SALL1) and Sin3 histone deacetylase corepressor complex component SDS3 (SUDS3 or SDS3), fused to the C-terminus of dCas9, and examine its efficacy across a range of applications and cell types. We demonstrate that chemically synthesized sgRNAs can be used in cells stably expressing dCas9-SALL1-SDS3 to achieve robust target gene repression that is consistently greater than the repression observed in cells expressing dCas9-KRAB. Furthermore, we show the efficacy of synthetic sgRNAs used with in-vitro transcribed dCas9-SALL1-SDS3 mRNA for robust CRISPRi-mediated repression. CRISPRi using synthetic sgRNA guides enables rapid characterization of gene function complementary to other loss-of-function methodologies and allows for the performance of complex end-point assays and the examination of a wide range of phenotypes in hiPSCs.

Keywords: CRISPRi, siRNA, Cas9

Poster: 112

TECHNOLOGY DEVELOPMENT INSIGHTS DERIVED FROM A TECHNO-ECONOMIC AND ENVIRONMENTAL ASSESSMENT OF COMMERCIAL CULTURED MEAT PRODUCTION

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Cultured or cultivated meat is genuine animal meat or seafood grown by cultivating animal stem cells in bioreactors without requiring animal slaughter. Cultivated meat holds tremendous potential to reduce the environmental impact of meat and seafood production, mitigate zoonoses and antibiotic use linked to animal agriculture, and provide food security for a growing population. A life cycle assessment and techno-economic analysis (commissioned by The Good Food Institute (www.gfi.org) and GAIA (www.gaia.be)) performed by CE Delft (www.cedelft.eu) modeled a large-scale cultivated meat production facility that operates in the year 2030 and produces 10,000 tons of ground meat per year. The reports find that cultivated meat production is likely to have reduced overall environmental impacts and be cost-competitive with some forms of conventional meat within the next decade. Insights from the report highlight key knowledge gaps and areas for technology development to further lower costs and environmental impacts of production. These areas include, but are not limited to, the establishment of cell line repositories to increase research accessibility, mapping the metabolism of the various cell types and species used in production, decreasing the cost of recombinant protein and growth factor production, recycling of medium components, optimization of large-scale perfusion bioreactors, and implementation of automation and continuous processing. Technologies created for cultivated meat production have implications for reducing costs and increasing productivity in other industries that rely on human or animal cell bioprocessing, such as regenerative medicine, cellular therapeutics, vaccines, and biologics. Synergistically solving shared challenges across these sectors promises to benefit all human and animal cell biomanufacturing industries.

Funding Source: Funding for the research was provided by generous philanthropic donations that power The Good Food Institute (www.gfi.org) and GAIA (www.gaia.be).

Keywords: cultured meat, cultivated meat, techno-economics

Poster: 113

TARGETED DNA-WRITING IS IMPROVED WITH DNA MINISTRINGS IN ANIMAL & PLURIPOTENT STEM CELL MODELS

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Digital genome engineering is a branch of synthetic biology which accelerates combinatorial genome modification of cells or organisms using DNA-writing and DNA-editing technologies and aims to increase the range of edit types, to simultaneously edit at multiple genomic loci, and to improve the speed and accuracy of on-target gene editing. Strategies to improve knock-in gene editing include chemical complementation, cell-cycle synchronization, extension of isogenic homology arm lengths and marker-assisted co-selection among others; nonetheless, there still is considerable room for improvement of seamless replacement by homology-directed repair. We and others have documented multi-copy head-to-tail targeted arrays, which present themselves as concatemers at the intended knock-in site using standard gene targeting reagents: double-stranded DNA plasmids as donor vectors that encode a repair template for knock-in with Cas endonucleases in ribonucleoprotein formats. We observe these multi-copy arrays at the intended target site regardless of cell type such as human or mouse pluripotent stem cells or human cancer lines, of animal model species including mouse, rat, pig and cow, of developmental stage such as zygotic or 2-cell pre-implantation embryos, or of delivery method including nucleofection, slide or cuvette electroporation or microinjection. We explore the hypothesis that DNA-end topology can improve on-target gene editing outcomes using linear covalently closed DNA repair templates that we refer to as DNA ministrings. Finally, we outline strategies for detecting and collapsing precisely targeted multiple integration events and for improving on-target DNA-writing to endeavor digital genome engineering.

Keywords: DNA ministrings, linear covalently closed double-stranded DNA, knock-in gene editing

Poster: 114

CONTROLLED AND SCALABLE T-CELL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELL DERIVED BLOOD PROGENITOR CELLS USING THE ENGINEERED THYMIC NICHE TECHNOLOGY

Woodside, Steven[1], Kirouac, Dan[1], Shukla, Shreya[1], Carpenedo, Richard[1], Pallaoro, Alessia[1], Hoi, Hiofan[1], Mohtashami, Mahmood[2], Trotman-Grant, Ashton[3], Zúñiga-Pflücker, Juan-Carlos[3], Zandstra, Peter[4]

[1]Research and Development, Notch Therapeutics, Vancouver, BC, Canada, [2]Biological Sciences, Sunnybrook Research Institute, Toronto, ON, Canada, [3]Department of Immunology, University of Toronto, ON, Canada, [4]School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada

Adoptive T- cell therapies are effective for some hematological malignancies and could transform the treatment of many other cancers. Despite early clinical success, use of genetically modified patient-derived T-cells poses challenges that limit the utility and accessibility of this cellular immunotherapeutic. T-cells derived from genetically modified, clonal, induced pluripotent stem cells (iPSC) can enable a defined, reproducibly manufacturable cell product. However, inducing T-lineage commitment in a scalable and controlled format suitable for clinical manufacturing remains a major obstacle. T-lineage commitment occurs in the thymus through Notch signaling activation in hematopoietic stem/progenitor cells (HSPC) by ligands such as Delta-like 4 (DL4) expressed on adjacent epithelial cells. In vitro, Notch signaling in HSPCs can be activated by co-culture with DL4-expressing cell lines, or by culture on DL4-coated tissue culture plates. Such systems lack control of the intensity and dynamics of Notch signaling needed for robust T-cell development (especially for iPSC derived HSPC) and are not easily scalable to meet the needs of clinical manufacturing. To address these limitations, we developed the Engineered Thymic Niche (ETN) platform, consisting of magnetic beads coated with DL4 +/- vascular cell adhesion molecule 1 (VCAM-1). This technology enables differentiation of iPSC-derived CD34+ HSPC to progenitor T-cells (ProT). Notch signaling was quantitatively and dynamically modulated, as measured via Hes1 expression and resultant T-lineage commitment based on CD5+CD7+ expression. A physicochemical-based computational model of ligand-receptor interactions at the bead-cell interface was developed to inform the design and dosing of the ETN beads. Model predictions for DL4:VCAM-1 ratios and Notch signaling dynamics guided experimental optimization of the differentiation process. Average outputs of 700 ProT per input iPSC CD34+ HSPC, with >80% ProT purity after 28 days in serum-free culture were measured. ETN beads were removed from culture prior to further maturation of the ProT cells using magnetic separation. The ETN technology provides a foundation for time and dose-dependent Notch signaling in a manner that enables repeatable manufacturing of iPSC-derived T cells.

Keywords: T cell therapy, In vitro T cell differentiation, T cell manufacturing

Poster: 115

HIGH FIDELITY CRISPR-CAS9 REAGENTS FOR STEM CELL EDITING IN HUMAN IPSCS

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CRISPR-Cas9 has become one of the most used gene therapy tools due to its simplicity and effectiveness. To improve the usefulness of the method for clinical and RUO applications, improvements to increase on-target editing efficiency and homology-directed repair (HDR) as well as reducing undesired off-target genome cuts are needed. We applied a NGS assay (TEG-seq) to screen several in-house engineered and published high-fidelity (HF) Cas9 variants. We identified a HF-Cas9 variant which outperformed others for improved specificity and functional activity. To further improve the on-target editing efficiency, especially insertion of SNPs or genes by HDR, we screened dozens of candidates that modulate DNA repair pathways. One candidate dramatically improves HF-Cas9 on-target HDR efficiency through the mechanism of extending and maintaining the single strand overhang at the double strand break site, forcing the cellular repair to favor HDR. We found that this enhancement of on-target activity does not increase off-target mutations. By using the HF-Cas9 and our enhancer we demonstrated this non-viral system can edit iPSC with high on-target and HDR efficiency and low off-targets on the sickle cell disease related HBB SNP site, and other genes (TRAC, TRBC, CD52, PD1) related to CAR-T cancer therapy. The enhancements to the CRISPR-Cas9 tool set will significantly benefit gene therapeutic research and clinical trials.

Keywords: CRISPR-Cas9, HiFi Cas9, off targets

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CRISPR/CAS9 GENE EDITING FOR GENERATING IPSC MODELS OF HUMAN DISEASES AND DEVELOPMENT AT INFINITY BIOLOGIX (IBX)

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Infinity Biologix (IBX, ibx.bio), formerly RUCDR, has provided the scientific community with the highest quality biomaterials, technical consultation, and logistical support. We collaborate with researchers, the NIH and non-profit foundations to provide stem cell services. These services include the banking and distribution of source cells and induced pluripotent stem cells (iPSC) and the generation of iPSC from human somatic cells. Since 2017, we have begun offering genetic engineering of iPSC harnessing the CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas9 technology. The diverse genetic background of the human subjects has hampered the usefulness of iPSCs for modeling human diseases and development. The use of the CRISPR/Cas9 system can create isogenic cell lines that will serve as better controls and help eliminate effects that are due to genetic variance rather than a biological mechanism. At IBX we have developed a high throughput, cost efficient workflow for using CRISPR/Cas9 to genetically modify iPSC from affected or unaffected subjects. Using this strategy, we have generated

footprint-free edited isogenic iPSC pairs harboring mutations involved in neurological disorders such as Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease and Huntington's Disease. All edited iPSC lines are tested rigorously for off-target effects, homogeneity, pluripotency and genetic integrity. Data of our quality control process is included in the COA for each iPSC line we distribute.

Keywords: CRISPR, iPSC, disease modeling

Poster: 117

PRECISION GENOME EDITING OF IPSCS USING CRISPR

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The ability to efficiently edit the genome of induced pluripotent stem cells to correct disease causing mutations hugely expands their utility in disease modeling, drug discovery and regenerative medicine. IPSCs offer the opportunity to obtain a diverse progeny in sufficient quantities for the study of molecular mechanisms and cellular processes dysregulated in genetic diseases. Here we describe a streamlined method to efficiently correct or insert clinically relevant mutations that allow the study of potentially every genetic disease. The approach includes the rational design of both single guide RNA and DNA repair oligo, the introduction of these components in iPSCs using the recombinant CRISPR/Cas9 system, single-cell cloning and screening for desired edits. We establish specific criteria for selecting sgRNAs with high predictive targeting efficiency and donor DNA that promote high homologous recombination. Donor oligos and single-guide RNAs are chemically synthesized and electroporated along with recombinant Cas9 into iPSCs. After 72 hours, half of the cells are collected for Sanger sequencing, while the other half are cryopreserved for single-cell cloning. Homologous recombination efficiency is calculated using the Interference for CRISPR Edits (ICE) tool from the Sanger sequencing of pooled electroporated cells. Once targeting efficiency is known, then cells are thawed, plated in 96-well format, and after 10 days, single-cell clones are screened for specific modifications. This method yields overall targeting efficiency ranging from 10 to 50% for single base change. This approach is efficient, robust, and reliable at generating isogenic iPSCs that allow the study of specific disease mechanisms.

Keywords: iPSCs, Crispr/cas9, Homologous Recombination

Poster: 118

SYNERGISTIC EFFECTS OF MECHANOTRANSDUCTION AND CHEMICAL CUES ON SCHWANN CELL DIFFERENTIATION OF DENTAL PULP STEM CELLS

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Current treatments of peripheral nerve injuries (PNIs) result in slow and inefficient regeneration, yielding poor clinical outcomes. The delivery of neurotrophic factors (NTFs), growth factors naturally produced by neural crest-derived Schwann cells (SCs), to sites of PNIs can enhance regeneration. Furthermore, guidance cues have been shown to orient axon extension towards the end organ, improving functional recovery. Dental pulp stem/progenitor cells (DPCs) have been shown to also highly express NTFs, likely due to their shared developmental origins with SCs. DPCs also have a propensity for differentiating towards an SC phenotype, further enhancing their NTF production. We have recently shown that DPCs can form scaffold-free cell sheets that act as NTF delivery vehicles and enhance regeneration when wrapped around PNIs in rats. The goal of this study is to now test if inducing SC-differentiation by the DPCs further enhances the bioactivity of our cell sheets and to evaluate if DPCs can form scaffold-free cell sheets with an aligned extracellular matrix (ECM), which is able to orient axon extension. To accomplish this, we tested the effect of culturing DPCs in an SC-differentiation media on NTF expression and evaluated the effect of growing DPCs on micro-grooved substrates on orienting ECM deposition. Immunofluorescent analysis showed that the resulting DPC sheets produced an aligned, collagenous ECM. Inducing SC differentiation increased the mRNA and protein expression of SC-markers (S100) and NTFs (brain-derived NTF, glial cell-derived NTF, and neurotrophin-3) compared to DPC sheets cultured on flat substrates, and this expression was further enhanced in the SC-induced cell sheets formed on the micro-grooved topography. This indicates that the chemical and mechanotransductive cues of our culture system worked synergistically to enhance the differentiation of DPCs toward a SC phenotype. Furthermore, we found that the DPC sheets were able to functionally induce and orient neurite extension in co-cultured neuronal cells in vitro. These aligned DPC sheets can be used to form bioactive nerve conduits capable of enhancing nerve regeneration by providing both a continuous supply of NTFs, to promote axon regeneration, and guidance cues from an aligned ECM, to direct axon extension.

Keywords: Dental Pulp Stem Cells, Schwann Cells, Neural Regeneration

Poster: 119

BRAIN ORGANOIDS: A NOVEL TOOL TO STUDY OXIDATIVE STRESS-INDUCED HUMAN CENTRAL NERVOUS SYSTEM DAMAGE FOLLOWING EXPOSURE TO IONIZING RADIATION

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Oxidative stress (OS) and the associated increases in inflammatory markers play major roles during development, aging, and progressive degenerative disease states of the Central Nervous System (CNS), including Alzheimer's disease, Parkinson's disease, and neurodevelopmental deficits. Ionizing radiation (IR) is one of many agents known to elevate OS through interactions with water and biomolecules, consequently producing pro-oxidants such as reactive oxygen species (ROS) and other free radicals. Increased levels of ROS could overwhelm antioxidant defenses which protect lipids, proteins, and DNA from OS, causing lasting changes to cell function and overall tissue morphology. Despite progress in understanding the roles of OS in CNS pathophysiology, studies using planar 2D neural cell cultures and animal models have been limited for their translational impact. However, three-dimensional (3D) brain organoid cell culture systems have emerged as novel models to simulate the organization and cell diversity of human brain regions in vitro. We propose brain organoids as a novel tool to study the cell-specific effects of IR on normal neural tissue as these models recapitulate features of the human brain, including self-directed 3D cytoarchitecure and functional activity. In this study, we will use brain organoids to characterize IR- and OS-induced changes to neural cell viability, morphology, differentiation, and secreted factors in the human cortex. Here, we deliver proton radiation at low-doses to characterize the acute responses of induced pluripotent stem cell-derived "cortical organoids". Based on our previous studies, we have selected protons due to their prevalence in the space environment, as well as their utility in cancer radiotherapy, where normal tissue may be at risk due to off-target effects. This research will evaluate changes to expression of proliferation, apoptotic, and cell-type specific markers using immunofluorescence and confocal microscopy. We will also evaluate changes to markers for lipid peroxidation and DNA damage. Results from this study will support the use of brain organoids as a translational tool to investigate OS-induced cellular and molecular changes potentially involved in progressive CNS neurodegenerative diseases as well as radiation-induced toxicity to the CNS.

Keywords: Oxidative Stress, Ionizing radiation, Neural organoids

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DMSO-FREE CRYOPRESERVATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS - CRYOBIOLOGICAL CHALLENGES, MECHANISMS OF ACTION, AND IMPLICATIONS FOR IPSC-BASED RESEARCH AND MANUFACTURING

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Induced pluripotent stem cells (iPSCs) are the backbone of stem cell research and its therapeutic endeavors. However, it has been a challenge, often overlooked, to bank iPSCs with high efficiency and consistency using conventional methods. We are introducing a new method for the cryopreservation of human iPSCs, in the form of cell aggregates, that was optimized by differential evolution and consists of only non-DMSO cryoprotective agents (CPAs). These molecules were found to act in concert to protect cells, including plasma membrane, cytoskeleton and organelles, from dehydration and ice. In contrast with single cells, multicellular aggregates were found sensitive to ice nucleation temperature of -8˚C and below, when they were frozen in DMSO. Cell recovery measured by membrane exclusion dyes immediately post-thaw was not indicative of iPSC survival in post-thaw culture. Using low-temperature Raman spectroscopy, a unique form of ice crystals was observed under the synergistic interaction of sucrose, glycerol, L-isoleucine and poloxamer 188 with water. Not requiring removal of the CPAs and upon wash-free thawing, iPSCs cryopreserved in this solution showed seeding efficiency in culture statistically comparable to fresh cells. Differentiation into all three germ layers and high-purity sensory neurons were successful using cryopreserved cells without additional subculture. In stress testing, the iPSCs in DMSO-free CPAs withstood 3 freeze-thaw-passage cycles with chromosomal and phenotypical stability. Most interestingly, this new formulation mitigated the iPSCs' sensitivity to undercooling and tolerated a wider range of ice nucleation temperatures, between -4 and -12˚C, bringing more flexibility and greater reproducibility to iPSC cryopreservation. Furthermore, ongoing study dissects the cryobiological changes, in membrane fluidity and membrane partitioning of CPA solutes for example, that cells undergo along neuronal differentiation and maturation. It is unveiling the directionality or predictable trend of such changes and observing a consistent cryoprotection of the DMSO-free formulation, which will establish the foundational knowledge in guiding upcoming cryopreservation designs for differentiated cells.

Funding Source: National Institutes of Health, Grant/Award Numbers 1R01EB023880 and 9R01HL154734.

Keywords: Cryopreservation, Non-DMSO cryoprotective agents, Low-temperature Raman spectroscopy

Poster: 121

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

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The Allen Institute for Cell Science has created a collection of endogenously tagged Human Induced Pluripotent Stem Cell (hiPSC) lines to illuminate cell organization. To date, the Allen Cell Collection consists of >40 single- or dual-edited lines that have undergone extensive quality control testing to ensure genomic, cell biological, and stem cell integrity. We have tagged many commonly recognized membrane-bound and membrane-less cellular organelles, signaling complexes, phase transition markers, transcription factors, and cardiomyocyte-specific structural markers. Our most recently released lines illuminate

chromatin-localized structures such as telomeres (TERF2-mEGFP), polycomb repressive complex 2 (EZH2-mEGFP) and RNA polymerase II (POLR2A-mEGFP). This work highlights our gene-editing and quality control workflows for mono- and biallelic editing of expressed or silent genes that are expressed during cardiomyocyte differentiation. Furthermore, we share updated protocols utilizing Adeno-Associated Virus (AAV) to deliver donor DNAs for gene tagging, enabling us to generate gene edited cell lines with greater efficiency. Using AAV as our repair vector in combination with RNP, we show preliminary results that have increased our HDR% 10-fold as compared to editing the same loci with a plasmid DNA donor. Further, we identified that transducing cells in the absence of pen/strep reproducibly increased HDR% 2-to-3 fold. 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: ENDOGENOUSLY TAGGED, CELL ORGANIZATION, Cardiomyocyte differentiation

Poster: 122

OPTIMIZED APPROACHES FOR GENE MODULATION AND GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Whether studying disease or normal human cell biology, primary human cell models are not always available and can be challenging to work with in the lab. Induced pluripotent stem cells (iSPCs) are a useful research tool because they are a renewable model for karyotypically normal cells. Additionally, they can be manipulated in the pluripotent state and differentiated into various isogenic cell types. The ability to manipulate the genome and modulate gene expression in these cells opens endless pathways for research. Horizon Discovery drives the applications of gene modulation and genome editing within the global life science market to support scientists on the path from research to therapy. Here we demonstrate tools and techniques for modulating gene expression in iPSCs using a variety of methods including RNAi, CRISPR-Cas9 knockout, CRISPR/Cas9 knock-in, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). Here, we show the techniques used to achieve functional knockout of SOX2 in iPSCs with efficiencies greater than 80%, knock-down of control gene PPIB to greater than 70% with CRISPRi and greater than 90% with RNAi, and over 100-fold activation of ASCL1 with CRISPRa. In addition to lentivirus options, our high-quality synthetic RNAs and mRNAs enable multiple methods to manipulate gene function with iPSCs in a DNA-free system.

Keywords: CRISPR, RNAi, iPSC

Poster: 123

ORGANOIDS FOR DISEASE MODELING AND IN VITRO DRUG SCREENING: AUTOMATED CULTURE MONITORING, IMAGING, AND ANALYSIS OF COMPLEX BIOLOGICAL SYSTEMS

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3D cell models representing various tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. While the complexity of 3D models remains a hurdle for the wider adoption in research and drug screening, significant progress has been made in developing tools for the automated culture and cell analysis. We describe an automated integrated system that allows automated monitoring, maintenance, and characterization of growth and differentiation of stem cells and organoids, as well as testing the effects of various compounds. The automated integrated system included the IXM-C imaging system, an automated incubator, a Biomek liquid handler, and a robotic device. We demonstrated methods for successful automation of three complex workflows: iPSC culture, 3D lung organoids, and intestinal organoids. iPSC's were automatically cultured, passaged, and monitored daily by imaging. Cell phenotypes, density, and colony sizes were automatically detected and characterized by AI-based image analysis, and the resulting data guided passaging steps. 3D lung organoids were developed from primary human lung epithelial cells cultured in Matrigel with a mixture of growth factors. Organoids were monitored using transmitted light, then stained and imaged in Matrigel using automated confocal imaging. Image analysis included conventional and AI-based tools. Developing organoids comprised spherical objects with complex morphology including cavities, protrusions, and vesical structures. Increase in size and complexity was monitored during 6-8 weeks of development. Advanced image analysis allowed 3D reconstitution and complex analysis of organoids, cell morphology, viability, and differentiation markers. We characterized multiple quantitative descriptors of organoids that could be used for studying disease phenotypes and compound effects. We measured concentration-dependent effects of several drugs that have been known to cause lung toxicity. Intestinal organoids were developed from primary mouse intestinal cells and were studied for effects of inflammatory cytokines. The workflow demonstrates the usefulness of automation and advanced high-content imaging for increased throughput and information in organoid assays, critical for compound screening.

Funding Source: No external funding

Keywords: 3D organoid models, high content imaging, cell culture automation

12:00 - 13:00 EDT

POSTER SESSION 1

TISSUE STEM CELLS AND REGENERATION

Poster: 135

HUMAN INFRAPATELLAR FAT PAD MESENCHYMAL STEM CELLS SHOW IMMUNOMODULATORY EXOSOMAL SIGNATURES

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Within the human knee infrapatellar fat pad (IFP) and synovium, resident synoviocytes and macrophages contribute to the onset and progression of inflammatory joint diseases. We have recently reported that a single intra-articular injection of human IFP-derived mesenchymal stem cells (IFP-MSC) reverses inflammation and fibrosis linked with macrophage polarization from an M1 in disease to an M2 phenotype following MSC therapy. Our hypothesis is that IFP-MSC robust immunomodulatory effects are largely exerted via their exosomal secretome by attenuating synoviocyte and macrophage proliferation and pro-inflammatory activation. Herein, we investigated human IFP-MSC derived exosomal (exoIFP-MSC) protein and miRNA signatures, and their effects on stimulated with inflammatory and fibrotic cues, synoviocytes and macrophages. exoIFP-MSC showed distinct miRNA and protein immunomodulatory profiles. Reactome analysis of 24 miRNAs highly present in exosomes showed their involvement in the regulation of six gene groups related to the: immune system, NGF/PDGF/Wnt pathways, cell cycle, gene expression, cellular responses to stress, and homeostasis. Exosomes were enriched for immunomodulatory and reparative proteins that are involved in positive regulation of cell proliferation, response to stimulus, signal transduction, signal receptor activity, and protein phosphorylation. TNFα/IFNγ/CTGF stimulated synoviocytes exposed to exoIFP-MSC demonstrated significantly reduced proliferation and secretion of pro-inflammatory molecules including IL-6, IL8, IP-10, MCP-1, MCP-2, RANTES compared to stimulated alone. Similarly, PMA/Ionomycin stimulated macrophages exposed to exoIFP-MSC showed significantly reduced proliferation and secretion of 22 pro-inflammatory molecules compared to stimulated alone. Also, their molecular profiling indicated a strong gene expression shift towards an M2 macrophage polarization. In conclusion, IFP-MSC show potent molecular and protein immunomodulatory exosomal profiles that significantly affect synoviocyte and macrophage functionality in inflammatory conditions in vitro. Based on these collective findings, we propose a viable cell-free alternative to MSC-based therapeutics as an alternative approach to treating synovitis and fat pad fibrosis.

Funding Source: Soffer Family Foundation

Keywords: Mesenchymal stem cells, Exosomes, Inflammation

Poster: 136

KNOCKDOWN OF CYSTEINE RICH SECRETORY PROTEIN LCCL DOMAIN CONTAINING 2 (CRISPLD2) AFFECTS DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (hMSCs) are promising tools for tissue engineering and regenerative medicine due to their multilineage differentiation potential. Long- term in vitro expansion of primary hMSCs is necessary to obtain sufficient cells for their therapeutic applications; however, prolonged expansion leads to impaired multipotency of hMSCs, which is a major roadblock for applications of the cells. To explore the underlying mechanism causing loss of differentiation capability, the differentially expressed genes between early and late passages of hMSCs were identified by RNA-sequencing and further confirmed by qRT-PCR. Knockdown of CRISPLD2 was achieved by transfection of hMSCs with siRNA. Osteogenic and adipogenic differentiation of hMSCs were induced by culturing cells in osteogenic and adipogenic medium followed by Alizarin Red Staining and Oil Red O staining, respectively. Cell proliferation was evaluated using alamarBlue assay. Senescence-associated β-galactosidase (SA-β-gal) staining was used to detect hMSCs senescence. We found 25 downregulated genes shared by late passage human Bone Marrow Stem Cells (hBMSCs), human adipose stem cells (hASCs), and human dental pulp stem cells (hDPSCs). Of them, Cysteine-rich secretory protein LCCL domain-containing 2 (CRISPLD2) was the most downregulated, with over 90% decrease in passage 11 compared to passage 3 cells. Knockdown of CRISPLD2 resulted in impaired osteogenic differentiation of hMSCs but increased adipogenic differentiation. This finding agreed with literature reporting that molecular regulation of osteogenic differentiation is opposite to adipogenic differentiation. Furthermore, we determined that CRISPLD2 knockdown did not affect cell proliferation or trigger cell senescence. In conclusion, CRISPLD2 plays a vital role in maintaining the homeostasis of hMSCs multipotency. Decreased CRISPLD2 expression in hMSCs during in vitro expansion can cause the cells to lose osteogenic differentiation ability.

Funding Source: This research was supported by grants from NIH/NIAMS and Louisiana Board of Regents.

Keywords: Cysteine-rich secretory protein LCCL domain-containing 2 (CRISPLD2), Osteogenic differentiation, Mesenchymal stem cells

Poster: 137

OXYTOCIN MEDIATES NEUROENDOCRINE REPROGRAMMING OF THE EPICARDIUM IN HEART REGENERATION

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Cardiovascular disease (CVD) is the leading cause of mortality both in the United States and worldwide and often results in the massive loss of cardiomyocytes, the chief contractile cells of the

heart. Critical work in the last two decades demonstrates that these lost cells can be partially regenerated by the epicardium, the outermost mesothelial layer of the heart, in a process that highly recapitulates its role in heart development. Upon cardiac injury, mature epicardial cells activate and undergo epithelial-mesenchymal transition (EMT) to form epicardium-derived stem cells (EPDCs), which are multipotent progenitors that can differentiate into several important cardiac lineages, including cardiomyocytes and vascular cells. In mammals, this process alone is often insufficient for significant regeneration, but it can be primed by specific factors in order to improve its efficiency. To that end, our group has recently discovered evidence that oxytocin (OXT), a hypothalamic neuroendocrine peptide, promotes a pro-regenerative phenotype in the heart. Here we show that OXT induces cellular proliferation and EMT in a model of human induced pluripotent stem cell (hiPSC)-derived epicardial cells, effects that are prevented after knockdown of its receptor. In addition, OXT is released from the brain into the bloodstream after cardiac cryoinjury in zebrafish, causing epicardial activation and heart regeneration. These processes are significantly impaired when OXT signaling is inhibited. Finally, oxytocin is also critical for proper epicardium development in zebrafish embryos. Our research reveals for the first time a direct brain-controlled mechanism that induces cellular reprogramming and regeneration of the injured heart, a finding that could yield significant translational advances in the treatment of CVD.

Keywords: Heart Regeneration, Epicardium, Oxytocin

Poster: 138

NEUREGULIN-1, IN A CONDUCIVE MILIEU WITH WNT/BMP/RETINOIC ACID, PROLONGS THE EPICARDIAL-MEDIATED CARDIAC REGENERATION CAPACITY OF NEONATAL HEART EXPLANTS

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Cardiac sympathetic nerves are required for endogenous repair of the mammalian neonatal heart in vivo, but the underlying mechanism is unclear. We tested the hypothesis that a combination of cardiac developmental growth factors Wnt3a, BMP4 and Neuregulin (NRG-1), compensate for denervation and support cardiac regeneration in explanted neonatal mammalian hearts. Hearts from 2-day old neonatal mice were harvested, lesioned at the apex and grown ex vivo for 21 days under defined conditions. Hearts grown in canonical cardiomyocyte culture media underwent complete coagulative necrosis, a process resembling ischemic cell death, by day 14. However, the addition of Wnt3a, BMP-4 and NRG-1, maintained cellular integrity and restored the endogenous regenerative program. None of these factors alone, or in any paired combination, were sufficient to induce regeneration in culture. rNRG-1 alone significantly reduced the accumulation of double strand DNA damage at Day 3; (–NRG-1: 60±12%; +NRG-1: 8±3%; $P<0.01$) and prevented coagulative necrosis at Day 14. Short-term addition of rWnt3a and rBMP-4 (day 0-3, NRG-1+) increased WT1 expression (a marker of epicardial cells) 7-fold, epicardial proliferation (78±17 cells vs. 21±9 cells; $P<0.05$), migration and recellularization (80±22 vs. zero cells; $P<0.01$; n=6) at the injury site on day 14. This novel explant culture system maintains three-dimensional (3D) neonatal mouse hearts and the mammalian neonatal cardiac regenerative program ex vivo. We identified that rNRG-1, plus short-term activation of Wnt- and BMP-signaling, promotes cardiac repair via epicardial cell activation, their proliferation and migration to the injury site, followed by putative cardiomyocyte recruitment. This novel technique will facilitate future studies of mammalian cardiac regeneration and may be useful in cardiac-specific drug testing.

Funding Source: none

Keywords: Cardiac Regeneration, Epicardium, Cardiac Explant Model

Poster: 139

ULTRA-STRUCTURAL STUDY OF THE HUMAN URINE DERIVED MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) are undifferentiated cells responsible for tissue homeostasis and repair. MSCs are readily isolated from the bone marrow, umbilical cord and adipose tissue. The discovery of urine derived stem cells (UD-SCs) introduced a leap in regenerative medicine applications. The non-invasive isolation of MSCs from voided urine offered an innovative source. However, the morphological and functional characteristics of the UD-SCs have not been properly addressed. In this work, we studied the ultrastructure of UD-SCs in relation to their functions using the transmission electron microscope (TEM). Their Phenotyping and differentiation capacity were also assessed. Results showed that UD-SCs express MSC surface markers including CD73, CD90, CD105 but not the hemopoietic markers CD14, CD34, CD45 and HLA-DR. UD-SCs expressed ICAM-1, an adhesion molecule specific for endothelial cells, known to mediate immune cell interaction. At passage five, UD-SCs revealed Alizarin red staining affinity in response to their differentiation into osteoblast. Toluidine blue stain evidenced their differentiation into chondrocytes, while the adipogenic differentiation was significantly impaired after passage three, as demonstrated by Oil Red stain. By TEM, ultrastructural indicators for differentiation were depicted as the cytoplasmic rosettes of calcium crystals in bone cells, the intercellular proteoglycan of cartilage matrix and the non-membrane bound lipid vacuoles for adipocytes. UD-SCs showed well-developed rough endoplasm, ribosomes and a secretory surface studded with polarized micro-plicae and micro-vesicles implying to their function as protein synthesizing cells. Abundant glycogen bodies were observed reflecting UD-SCs high energy stores relevant to their functional activity. Apart from confirming the MSC nature of UD-SCs, our results highlighted the peculiar expression of ICAM-1 warranting further functional study of the UD-SCs.

Keywords: urine derived stem cells, ultrastructure, Phenotyping and differentiation capacity

Poster: 140

EXTRACELLULAR MATRIX SIGNALING REGULATES PANCREAS MORPHOGENESIS AND ENDOCRINE CELL DIFFERENTIATION VIA A MECHANO-NOTCH SIGNALING AXIS

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The extracellular matrix (ECM) plays important roles in regulating many aspects of in vivo pancreas development. We have previously shown that cell-ECM interaction is required to initiate pancreas branching via Integrin beta1 (Itgb1, a principal ECM receptor); but whether pancreas cell differentiation also depends on ECM signaling remains unclear. In this study, we show that the deletion of Itgb1 in the developing pancreas (Itgb1ΔPan/ΔPan) results in the severe reduction of endocrine cell population at embryonic day (E)15.5. The expression of pro-endocrine marker Neurog3 was significantly reduced in Itgb1ΔPan/ΔPan pancreata, suggesting these pancreata fail to initiate the pro-endocrine differentiation program. Further analysis revealed that the expression of Notch signaling component Hes1, a Neurog3 repressor, was upregulated in E15.5 Itgb1ΔPan/ΔPan pancreata. Furthermore, blocking Notch signaling restored the expression of Neurog3 and endocrine cell differentiation in Itgb1ΔPan/ΔPan pancreas explants. These results suggest that the endocrine population decrease is due to Notch signaling mis-regulation when ECM signaling is perturbed. Because Notch signaling has been linked to mechano-dependent regulation of YAP, we next investigated whether mechano- and YAP signaling axes are affected in Itgb1ΔPan/ΔPan pancreas. Atomic force microscopy analysis revealed a significant increase in tissue stiffness of E13.5 Itgb1ΔPan/ΔPan pancreata. In accordance with tissue stiffening, Itgb1ΔPan/ΔPan pancreata exhibited an increase in Yap activity. Together, we reason that ECM-Itgb1 signaling controls tissue stiffness, which couples YAP and Notch signaling to coordinate pancreas morphogenesis and endocrine cell differentiation. Furthermore, utilizing an in vitro human embryonic stem cell (hESC) differentiation culture to pancreatic endocrine precursors, we also demonstrate the significant effects of microenvironment stiffness on cell fate. Differentiation of hESCs on soft scaffolds showed increased Neurog3 expression and decreased Hes1 expression as compared to hESCs on stiff scaffolds. Overall, the present study provides insights into how ECM-pancreatic progenitor cell interaction integrates the mechanotransduction and Notch pathways to orchestrate pancreatic cell fate decisions.

Funding Source: This work was supported by the JDRF Postdoctoral Fellowship Award 2019-742-A-N to M.M. and NIH 1R01DK119590 grant to H.P.S.

Keywords: Development, Mechanobiology, Pancreas

Poster: 141

GENERATION OF PATTERNED KIDNEY ORGANOID THAT RECAPITULATES ADULT KIDNEY COLLECTING SYSTEM FROM EXPANDABLE HUMAN/MOUSE URETERIC BUD PROGENITORS

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Current kidney organoids model development and diseases of the nephron but not the contiguous epithelial network of the kidney's collecting duct (CD) system. Here, we report the generation of an expandable, 3D branching ureteric bud (UB) organoid culture model that can be derived from primary UB progenitors from mouse and human fetal kidneys, or generated de novo from human pluripotent stem cells. In chemically-defined culture conditions, UB organoids generate CD organoids, with differentiated principal and intercalated cells adopting spatial assemblies reflective of the adult kidney's collecting system. Aggregating 3D-cultured nephron progenitor cells with UB organoids in vitro results in a reiterative process of branching morphogenesis and nephron induction, similar to kidney development. Applying an efficient gene editing strategy to remove RET activity, we demonstrate genetically modified UB organoids can model congenital anomalies of the kidney and urinary tract (CAKUT). Taken together, these platforms will facilitate an enhanced understanding of development, regeneration, and diseases of the mammalian collecting system.

Keywords: Kidney progenitor cells, Kidney organoids, Human pluripotent stem cells

Poster: 142

GENETIC APPROACHES TO ATTAIN HYPOIMMUNOGENIC HUMAN STEM CELL DERIVED BETA CELLS

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Type 1 diabetes (T1D) is an autoimmune disorder leading to the selective destruction of insulin-producing β-cells in the pancreas. Despite recent scientific advances, questions remain regarding the initial trigger and the downstream mechanisms of disease progression. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) provide new opportunities for cell replacement therapy of T1D. Therapeutic quantities of human stem cell-derived β-cells (SC-β) can be

attained in vitro following a stepwise differentiation protocol. Yet, preventing immune rejection of grafted cells, without the use of life-long immunosuppressants, remains a major challenge, and the optimal goal is to transplant naked cells that are genetically modified to evade the immune system and induce tolerance. Using T1D patients' hiPSC derived β-cells (iPSC-β), we developed a human in vitro platform in an autologous setting that recapitulates aspects of the effector/target interactions in an autoimmune response. We performed a droplet based single-cell RNA sequencing (scRNA-seq) of T1D iPSC-β, co-cultured with their autologous perihelial blood mononuclear cells (PBMCs). scRNA-seq data analysis of co-cultured cell populations identified upregulated genes that contribute to the initial inflammatory stimulation of T-cells, typical to the immune infiltrated pancreatic islets in T1D. Subsequent co-culture experiments have shown that CRISPR-depletion of such genes in SC-β, can reduce activation of T-cells and increase β-cell survival. These results unfold some insights into the nature of immune destruction of β-cells during T1D and provide means to prevent both autoimmune and allogeneic rejections of transplanted SC-β cells.

Keywords: Type 1 diabetes, Autoimmunity, Immune tolerance

Poster: 143

MICROVASCULATURE REGENERATION IN BETA CELL REPLACEMENT THERAPIES FOR THE TREATMENT OF TYPE 1 DIABETES

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Islet transplantation for the treatment of type 1 diabetes relies on the availability of appropriate pancreata and the transplantation success is challenged by poor engraftment due to the lack of vascularization and instant blood mediated inflammatory response (IBMIR). Therefore single-donor transplantation is not effective at controlling glycemia. We used a multi-disciplinary approach for vascularization of stem cell-derived pancreatic cells in the subcutaneous space, addressing donor scarcity, ischemia and IBMIR concurrently. Human embryonic stem cells derived pancreatic progenitors (PP) were transplanted subcutaneously in diabetic immunocompromised mice alone or with adipose-derived rat microvessels (MV), fragments with intact vascular lumen and perivascular cell coverage, or with dissociated endothelial cells (EC). At 1-week post-transplantation, MV connected with the host, becoming blood perfused which resulted in a significant decrease in cell death. The recipients of PP+MV, reached normoglycemia 7-8 weeks post-transplantation which is significantly faster than PP recipients in this study and previous studies. The PP+EC recipients failed to normalize glycaemia suggesting the superiority of using intact MV fragments compared to single EC. The PP+MV grafts responded to glucose challenge at week 7. Interestingly, the PP grafts responded to glucose challenge ex vivo, highlighting the importance of effective vascularization for in vivo functionality. Further, co-transplantation of human islets with MV led to successful islet engraftment in the subcutis resulting in immediate normoglycemia with subtherapeutic islet doses. Moreover, a dense intraislet vasculature was formed, resembling that of native islets. Therefore, using MV, leads to effective engraftment and accelerated graft function by minimally invasive subcutaneous transplantation.

Funding Source: Medicine By Design, Juvenile Diabetes Research Foundation, Toronto General Hospital Research Institute

Keywords: Type 1 Diabetes, Beta Cells, Microvessels

Poster: 144

MACHINE LEARNING METHOD IDENTIFIES HUMAN TISSUE-RESIDENT STEM CELLS FROM SINGLE-CELL DATA IRRESPECTIVE OF TISSUE-TYPE AND IDENTIFIES A NOVEL TISSUE STEM-CELL-ASSOCIATED GENE.

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Tissue resident stem cells play essential roles in tissue homeostasis in health and in disease and are targeted in many therapies. However, they are characterized by high biomolecular heterogeneity, and for this reason, the common tissue stem cell biology is difficult to define and study. In this study, we integrate machine learning with the specific biology of particular tissue-resident stem cells in uncovering generalized stem cell transcriptomic signatures. Using human single-cell transcriptomic data from the European Nucleotide Archive, individual cells from 9 healthy samples (7 organs, 133,640 cells) were labeled to be stem cells or non-stem cells based on a custom combination of experimentally validated stem-cell markers for each tissue-type. Using this method, tissue-resident stem cells could be distinguished from non-stem cells (balanced test accuracy of 0.88, AUROC of 0.95) irrespective of inter-organ heterogeneity. Linear methods perform well for non stem cells, but poorly for stem cells, highlighting stem cell complexity. Shallow multi-layered perceptrons performed the best in distinguishing stem cells from non-stem cells and using only the landmark 1000 genes did not reduce performance. Interestingly, we uncovered a modular network of receptors (ERBB2, ERBB3, NOTCH1) and transducers (SOX2, RPS6, RPS5) to be important in tissue-resident stem cells across several organs. The modular architecture of this network suggests that a small set of regulators may be combined uniquely in different organs. Finally, we identify a previously uncharacterized gene, LOC112694756, which was identified to be important and significantly upregulated in stem cells of all studied organs. Together, we report the innovative application of machine learning to study and identify tissue resident stem cells and identify a novel gene with a possible role in tissue-resident stem cells irrespective of organ localization. Following experimental application, this study bears significance for the field of regenerative medicine and stem cell biology.

Keywords: Tissue stem cells, Machine learning, Stem cell signaling

Poster: 145

SINGLE-CELL ANALYSIS IDENTIFIES A POPULATION OF LIVER PROGENITOR CELLS DURING REGENERATION

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The liver is an essential organ, responsible for key metabolic functions, energy storage, blood detoxification, plasma protein secretion, and bile production. It is composed of several different cell types and is capable of regenerating by replacing hepatocytes lost due damage, primarily through division of other hepatocytes. However, there are limits to the capacity of the liver to compensate following sustained insults or injury. Liver failure is one of leading causes of death worldwide, and the only known cure for liver failure is organ transplantation. The development of a stem-cell based therapy to regenerate the liver would provide a life-altering treatment for patients with late-stage liver disease. While there is some evidence for a transient population of liver stem cells that appear during regeneration, the origin, potency and regulation of these cells remains largely unknown. The zebrafish (Danio rerio) liver serves the same function and is thought to be composed of the same cell types as in mammals. To identify the response of the liver to acute injury on a cellular and transcriptomic level, the livers of adult zebrafish were subjected to either drug-induced toxicity or genetic hepatocyte ablation, and were subsequently analyzed using single-cell RNA sequencing. This approach resulted in the generation of transcriptomic information for ~25,000 single cells, inclusive of all known cell types in the liver. Intriguingly, a population of cells that contains markers for both hepatocytes and biliary epithelial cells appears early only after recovery from hepatocyte ablation. These data suggest a progenitor population of biliary origin is utilized during regeneration from extreme injury. Lineage tracing of adult biliary epithelial cells confirms that they can give rise to hepatocytes after hepatocyte ablation. Further work will be needed to characterize the regulation of these cells during regeneration.

Funding Source: I.M.O. is supported by the NIAAA (F32AA027135).

Keywords: single-cell sequencing, regeneration, stem cells

Poster: 146

A SERUM-FREE, BOVINE PITUITARY EXTRACT (BPE)-FREE MEDIUM SUPPORTING LONG-TERM FEEDER-FREE EXPANSION OF HUMAN PRIMARY KERATINOCYTES THAT RETAIN THEIR AIR-LIQUID INTERFACE DIFFERENTIATION POTENTIAL

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Human primary keratinocytes (HPKs) derived from the skin typically undergo growth arrest at approximately 15 - 30 population doublings (PDs) when maintained in traditional feeder-free culture medium formulations. To circumvent this early senescence, we developed DermaCult™, a serum-free, BPE-free medium that promotes long-term growth (> 30 PDs) of HPKs while maintaining their air liquid interface (ALI) differentiation potential. To measure cell expansion over the entire lifespan of the culture, commercially supplied HPKs derived from either neonatal or adult skin were thawed and seeded onto uncoated tissue cultureware at a density of 1 - 2.5 x 103 cells/cm2. Full-medium changes were performed every other day and cells were enzymatically dissociated and passaged when cultures reached approximately 50 - 70% confluence. At various time points, keratinocytes were analyzed by qPCR and flow cytometry to detect expression of basal and differentiated cell markers. Keratinocytes were also differentiated by increasing the calcium concentration when the cells were in conventional monolayer culture or in ALI culture. Keratinocytes derived from either neonatal or adult skin and cultured in DermaCult™ proliferated between 50 - 70 and > 70 PDs before undergoing senescence, respectively (n=4 for each cell type). Keratinocytes predominantly maintained a cobblestone morphology, expressed basal cell markers (TP63, KRT14, ITGA6), and were devoid of differentiation markers KRT10 and LOR (n=4). Induction of differentiation resulted in the expression of KRT10, LOR, and formation of ZO-1 tight junctions, with concomitant down-regulation of basal markers TP63 and KRT14. In ALI culture, HPKs (at 30 PD) generated a stratified epithelium including the stratum corneum, with the appropriate cellular architecture and expression of markers (TP63, KRT14, KRT5, KRT10, LOR, IVL) similar to their in vivo counterparts. In summary, we have developed an improved BPE-free medium for human primary keratinocyte culture that promotes greater expansion of HPKs and maintenance of their differentiation potential at later passages.

Keywords: Keratinocytes, Expansion, Long-term

Poster: 147

HIGHLY EFFICIENT DERIVATION AND EXPANSION OF GASTRIC ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS IN VITRO

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Derivation and expansion of gastric organoids from human pluripotent stem cells (hPSCs) represents an attractive tool for drug screening, disease modeling, and cell replacement therapy. We have developed STEMdiff™ Gastric Organoid Kit, which includes specialized serum-free media that promote differentiation of hPSCs through the developmental stages of definitive endoderm, posterior foregut, and stomach. Monolayers were generated from 2 human embryonic and 3 induced pluripotent stem cell lines maintained in mTeSR™1 on Corning® Matrigel®, then induced to differentiate to definitive endoderm. These monolayer cultures were then further differentiated to posterior foregut endoderm that buds off three-dimensional spheroids into the culture medium which can be harvested. These gastric progenitor spheroids express SOX2 and E-CADHERIN epithelial markers and the mesenchymal marker VIM. When spheroids are embedded in Matrigel® and

cultured in STEMdiff™ Gastric Organoid Medium, they mature into gastric organoids composed of a polarized epithelium and a surrounding mesenchyme. Gastric organoids differentiated for > 34 days consist of gastric pit cells (MUC5AC+), gland cells (MUC6+), G cells (GASTRIN+), endocrine cells (SST+), and progenitor cells (SOX2+, SOX9+, PDX1+). Organoids can also be passaged long term (> P10) in STEMdiff™ Gastric Organoid Expansion Medium or cryopreserved at any passage. Organoids cultured for multiple passages (> P3) maintain gastric gland cells (MUC6+), G cells (GASTRIN+), chief cells (PGA+, PGC+), stem cells (LGR5+), and progenitor cells (SOX2+, SOX9+, PDX1+). Cells of these organoids also express markers of proliferation (Ki67+) and tight junctions (CLD18+). In addition, apical-out gastric organoids express ACE2, making them susceptible to SARS-CoV-2 infection in vitro. In summary, STEMdiff™ Gastric Organoid Kit supports the highly efficient and reproducible derivation of gastric organoids from hPSCs.

Keywords: Gastric Organoids, Stomach Organoids, human pluripotent stem cells (hPSCs)

Poster: 148

THE SINGLE-CELL ATLAS OF HUMAN FETAL SALIVARY GLAND DEVELOPMENT PROVIDES NOVEL INSIGHTS INTO DEVELOPMENTAL BRANCH POINTS

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Multiple pathologies and non-pathological factors can disrupt salivary gland function including cancer and cancer therapeutics, autoimmune diseases, infections, pharmaceutical side-effects, and traumatic injury. Despite the range of pathologies, there do not yet exist definitive therapeutic or regenerative approaches to address salivary gland loss, likely due to significant gaps in our understanding of salivary gland development. Here we present the first single cell human fetal salivary gland atlas. Our analyses have revealed distinct populations of early-stage progenitors that independently give rise to different parts of the organ. Early epithelial progenitors give rise to two populations, one that gives rise to temporally distinct excretory duct and myoepithelial populations, and another that gives rise to striated and intercalated duct. Our data also suggests that ductal progenitors that give rise to intercalated and striated duct are more developmentally similar to striated duct, suggesting that the program that controls the bifurcation into these two lineages is controlled by the suppression of the striated duct gene program in order to give rise to intercalated duct. Importantly, though aspects of our analysis overlap with existing mouse developmental studies, for example, the importance of FGF10 and TFCP2L1 as developmental regulators, our studies have identified species-specific molecular signatures in human developing glands. Although mouse studies have identified Krt14 as a marker for early basal cell populations, our data shows no expression of KRT14 and showing instead that KRT15 marks those groups. Additionally, while c-Kit has been shown to mark intercalated duct, our data reveals very limited expression of KIT that is not limited to intercalated duct. Our studies introduce new human-specific developmental paradigms for salivary gland and lay the groundwork for the development of translational human therapeutics and ongoing developmental studies.

Keywords: salivary gland, single cell sequencing, fetal development

Poster: 149

SINGLE-CELL RNA SEQUENCING UNCOVERS RAPID SHIFT IN THE CELLULAR ARCHITECTURE OF THE MURINE TYMPANIC MEMBRANE AFTER INJURY

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Despite having a high prevalence, the underlying cellular interactions that govern tympanic membrane (TM) perforation regenerating are mostly unknown. Upon injury, the murine TM displays a robust increase in proliferation and rapid shifts in cellular behavior, but the identities and molecular mechanisms of these shifts are largely uncharacterized. This study aimed to transcriptionally define the cell populations activated in response to injury and during healing of the murine TM. TM perforations were made in both ears of adult mice, which were sacrificed 1, 3, 7, or 14 days following injury (n = 10 ears per timepoint). Cells were enzymatically dissociated and submitted for single-cell RNA sequencing (scRNA-seq) using the 10x Genomics platform. Two clustering algorithms, Seurat and CellFindR, were employed to identify clusters of cells, and the resulting gene expression matrices were queried to transcriptionally characterize each population. Over 10,000 cells were captured per time-point (range: 10,292- 22,366). Unsupervised clustering of the cumulative data revealed 97 total sub-populations of cells within 15 distinct top-level populations. Further analysis demonstrated the emergence of cellular subtypes unique to the injured and healing state as early as 1 day after perforation. The transcriptional data shows induction of distinct sub-populations of cells present from 1 day to 7 days post-injury, which then resolve by 14 days post-injury. Among the distinct cell shifts identified was a gross transcriptional shift among the stem/progenitor keratinocyte population after injury and the emergence of a distinct “wounded” keratinocyte progenitor population. At later timepoints, large populations of more differentiated keratinocytes emerge. Unlike the keratinocyte population, distinct reactive subpopulations of mucosal and mesenchymal cells do not emerge until 3 days following injury. Overall, this study defines the transcriptional and cellular architecture of the TM in homeostasis and regeneration. Key markers of these cell populations were identified and can be used for future investigation of the mechanism of wound resolution of the TM.

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Keywords: regeneration, injury, single-cell

Poster: 150

CHARACTERIZATION OF HUMAN BREAST CANCER STEM CELLS SUBPOPULATIONS BASED ON SIMILARITY TO NORMAL MAMMARY CELLULAR DIFFERENTIATION

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Cancers reflect the cellular diversity found in the normal tissues from which they originate. This diversity includes stem and progenitor-like cells called “cancer stem cells” (CSC) in malignant tissues. Because CSCs often resist standard cancer treatments and can remake a tumor after a clinical intervention, the characterization of CSCs is of high interest for tumor prognosis and for targeted therapeutic development. Scientists identified breast CSCs a long time ago. However, recent findings, including full transcriptomic analysis, have shown that breast CSCs isolated by different criteria or markers have different phenotypes, suggesting that different subpopulations of CSCs could exist, even within the same tumor. We hypothesize that i) subtypes of breast cancers originate from different populations of breast cancer stem cells and ii) that these populations of CSCs come from specific normal breast stem/progenitor cells. Because molecular information on normal human breast cells is sparse, we analyzed published RNA sequence data from normal mouse mammary tissue and identified cell surface markers that could help identify human breast CSC subpopulations. We validated those mouse markers by RT-qPCR and flow cytometry in human breast cancer cell lines representing all clinical breast cancer subtypes, classified according to the expression or non-expression of estrogen, progesterone, and HER-2 cell receptors. We evaluated the following cell lines: MCF-7, BT-474, SK-BR-3, MB-321, MB-468, and Hs 578T. Interestingly, we found that some of the newly identified markers were preferentially expressed only in some of the breast cancer subtypes. Furthermore, markers such as PROCR, CD14, and CD81, made it possible to distinguish subpopulations with negative or positive expression of these markers, suggesting the identification of unique cellular subpopulations. After identifying the most promising markers, we sorted cells and performed the mammosphere formation assay and RT-qPCR to identify stemness properties and stemness-related gene expression. In summary, we characterized new cell populations within human breast cancers and show that RNA sequence data from mice can be used as a starting point to study human breast cancer stem cells.

Keywords: Breast Cancer, Cancer Stem Cells, Cancer and Development

Poster: 151

PROTEASOMES IN HUMAN CULTURED AUTOLOGOUS ORAL MUCOSAL EPITHELIAL CELL SHEET

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Cultured autologous oral mucosal epithelial cell sheet (CAOMECS) have been successfully used as a non-limbal cell source to reconstruct corneal epithelium in case of bilateral limbal stem cell deficiency (LSCD). Further characterization of proteasome population in CAOMECS is needed to determine the role played by the constitutive proteasome, compared to the role played by the immunoproteasome (IPR) in the regenerative capacity of CAOMECS. Previously, we showed that CAOMECS have more than one proteasome population, while corneal epithelial cells (CEC) have only one proteasome subtype. CAOMECS were produced using donated oral mucosal epithelial biopsies obtained from research subjects, following informed consent procedures. NIH3T3 feeder cells and fetal bovine serum were used for cell sheet engineering. CAOMECS cell lysates were examined using mass spectrometry analysis and compared to cultured human corneal epithelial cells (CEC). Results showed that all alpha type subunits were highly expressed in CAOMECS. Catalytic core beta subunits were variously expressed, B6 and B5 were down regulated and B1, 2, 3, 4 and 7 were highly expressed in CAOMECS. However, the most dramatic increase was found in the inducible beta subunits B1i, B2i and B5i that make up the immunoproteasome. The subunits PA28A and PA28B of the regulatory complex that activates the IPR, were more expressed in CAOMECS than in CEC. Human leukocyte antigen HLA-B was also significantly high in CAOMECS. These results indicate that CAOMECS comprise a different type of proteasome population as compared to CEC. In conclusion, our results demonstrated that IPR pathway is activated and could play a major role in the proliferative and regenerative capacities of CAOMECS. Understanding the mechanism of action of each proteasome subtype distribution in CAOMECS will help to design an efficient and safe oral mucosal epithelial cell construct and improve the regenerative capacity of CAOMECS.

Funding Source: Support by Emmaus Medical, Inc.

Keywords: Immunoproteasome, Epithelium, CAOMECS

Poster: 152

WHEN DOES HUMAN TISSUE DEVELOP INTO A SENTIENT HUMAN BEING?: UTILIZING THE EMERGENCE OF CONSCIOUSNESS AS AN ETHICAL FRAMEWORK TO GROW HUMAN ORGANOGENIC PODS FOR SOLID ORGAN TRANSPLANTATION

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CRISPR technology has the potential to revolutionize personalized medical care and medical science. For example, personalized human, clinical-grade stem cells are likely capable of CRISPR modification such that, if provided a proper culturing environment, these cells can each be grown into what I call human organogenic pods. Once we have developed our proof-of-concept culturing system, we intend to develop human organogenic pods that will contain all of the transplantable solid organs of the thoracic and abdominal cavities. This is not simply science fantasy: the technologies are closer than some might think. In developing this thought experiment, I identified a critical difference between human tissue and sentient human beings: the emergence of consciousness. Consciousness itself is simply defined here as the ability of an organism to be aware

of itself or its environment. This review will identify evidence for the time frame when consciousness likely arises during human development and will potentially propose additional experiments as well. Initial reviews of existing data indicate that human consciousness most likely emerges immediately after birth. This time frame can help to provide guidance to science policy panels in establishing reasonable ethical boundaries for conducting developmental human tissue research, in particular for conducting experiments with developing organogenic pods in vitro. We thus are raising these issues now most likely to request an expansion of the existing 14-day rule (pending our full investigation of the consciousness and morphological data). This expansion of the time frame to grow organogenic pods will allow us an adequate amount of time during our proof-of-concept studies to identify at least the morphological differences between developing organogenic pods in vitro versus developing human fetuses in utero. In summary, these studies will provide an initial time frame for when human consciousness emerges. This knowledge will prevent an erroneous development of a conscious human being in vitro while ensuring we can experiment with the growth of quality organogenic pods for personalized, transplantable organs.

Keywords: Solid Organ Transplant, Human Organogenic Pods, CRISPR

Poster: 153

RETINAL ORGANOIDS ON-A-CHIP: A 3D PRINTED MICRO-MILLIFLUIDIC BIOREACTOR FOR LONG-TERM RETINAL ORGANOID MAINTENANCE

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Retinal degeneration is a leading cause of vision impairment and blindness worldwide and treatment for the advanced retinal disease does not exist. Stem cell-derived retinal organoids (RtOgs) are emerging approaches for tissue replacement therapy. However, existing RtOg production methods are highly heterogeneous. Controlled and predictable methodology and tools are needed to standardize RtOg production and maintenance. In this study, we designed a shear stress-free micro-millifluidic bioreactor for long-term retinal organoid culture and maintenance. We used a stereolithography (SLA) 3D printer to fabricate a mold from which Polydimethylsiloxane (PDMS) was cast. The fabrication methods effectively combined micro and millimeter features with low cost and rapid manufacturing. We optimized the chip design using in silico simulations and in vitro evaluation to optimize mass transfer efficiency and concentration uniformity of media in each culture chamber. We successfully cultured RtOgs on an optimized bioreactor chip for 37 days. We also qualitatively and quantitatively compared the RtOgs produced by conventional static dishes and our novel chip culture methods. Phase-contrast imaging showed that both conventional and chip-cultured RtOgs developed a transparent outermost surface layer. Fluorescence lifetime imaging (FLIM) showed that chip-cultured RtOgs had a significantly lower long lifetime species (LLS) ratio than static cultured ones, which demonstrated that the former exhibited less oxidative stress. RtOgs in bioreactor culture demonstrated higher NADH signal overall, but both bioreactor and conventional cultures showed similar free/bound NADH ratio over time, which indicated a normal differentiation time course. RtOg gene expression was examined by fluorescence imaging and quantitative polymerase chain reaction (qPCR) analyses. RtOgs in both groups showed thick nuclear outer layers expressing CRX on day 120. The gene profiling on both groups showed expressed retinal progenitor genes and most of the tested photoreceptor markers. In summary, we validated an autonomous micro-millifluidic device with significantly reduced shear stress and lower oxidative stress to produce RtOgs of equal or greater quality than those maintained in conventional static culture.

Funding Source: California Institute of Regenerative Medicine (CIRM) grant number TRAN1-10995; RPB unrestricted grant to UCI Department of Ophthalmology; ICTS KL2 Grant number is KL2 TR001416.

Keywords: Retinal organoids, 3D printing microfluidics, Fluorescence lifetime imaging

Poster: 154

CHARACTERIZATION OF THE HEMATOPOIETIC STEM/PROGENITOR CELLS AND THE BONE MARROW MICROENVIRONMENT IN SICKLE CELL DISEASE PATIENTS

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In Sickle Cell Disease, the periodic vaso-occlusive episodes initiated by reactive sickle cells result in a chronic status of inflammation in multiple organs. Little is known about the impact of SCD-induced inflammation in the bone marrow (BM) of Sickle Cell Disease (SCD) patients. In this study, we characterized the hematopoietic stem and progenitor cells (HSPC) and the stromal cells from BM aspirates of nine SCD patients before transplant in comparison with age-matched BM aspirates from healthy individuals. Using multiparametric flow analysis, we observed a group of SCD patients (n=5) with CD34+ frequency similar to controls, and a distinct group of SCD patients (n=4) that exhibited lower average frequencies of CD34+, HSCs, multipotent, and lymphoid progenitors cells compared to controls. We examined the transcriptomic landscape of the HSCs in the BM of SCD patients by single cell RNA-sequencing (scRNA-seq). Our analysis revealed a common SCD signature including several upregulated genes related to cell proliferation and differentiation such as FOS and CDK6; we also found several downregulated genes related to cell-cell signaling such as AVP and AREG in the HSC subset. Analysis of the BM stromal component showed a trend for increased non-hematopoietic cells including CD45-/CD31+ and CD45-/CD105+ subsets in SCD patients compared to controls, suggesting a change in their BM stromal niche.

In vitro, SCD BM-derived stromal cells revealed distinct gene expression patterns including increased inflammatory cytokines and inflammatory regulators. BM biopsies revealed alterations in the stromal components as evidenced by an overall decrease in cellularity in some patients. These observations were accompanied by an increase in angiogenic factors in the BM plasma of most patients. Taken together, these preliminary data suggest that SCD contributes to molecular and functional changes of hematopoietic cells and their BM niche and that the intensity of these changes may be associated with disease severity.

Keywords: scRNA-seq, CD34+ cell, sickle cell disease, bone marrow niche, bone marrow microenvironment, stem cells, bone marrow biopsy, scRNA-seq, CD34+ cell, sickle cell disease, bone marrow niche, ribosomal imbalance

Poster: 155

RNA-BINDING PROTEIN IGF2BP1 MAINTAINS LEUKEMIA STEM CELL PROPERTIES BY REGULATING HOXB4, MYB, AND ALDH1A1

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Insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) is an oncofetal protein expressed in various cancers including leukemia. In this study, we assessed the role of IGF2BP1 in orchestrating leukemia stem cell properties. Tumor-initiating potential, sensitivity to chemotherapeutic agents, and expression of cancer stem cell markers were assessed in a panel of myeloid, B-, and T-cell leukemia cell lines using gain- and loss-of-function systems, cross-linking immunoprecipitation (CLIP), and photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) techniques. We report that genetic or chemical inhibition of IGF2BP1 decreases leukemia cells' tumorigenicity, promotes myeloid differentiation, increases leukemia cell death, and sensitizes leukemia cells to chemotherapeutic drugs. IGF2BP1 affects proliferation and tumorigenic potential of leukemia cells through critical regulators of self-renewal HOXB4 and MYB and through regulation of expression of the aldehyde dehydrogenase, ALDH1A1. Our data indicate that IGF2BP1 maintains leukemia stem cell properties by regulating multiple pathways of stemness through transcriptional and metabolic factors.

Funding Source: NIH grant R01 AR063361 (VSS), NIH Intramural Research Program of the NIAID (SAM), and NIAMS (MH).

Keywords: Leukemia initiating cell, RNA-binding protein, IGF2BP1

Poster: 156

BONE MARROW INNERVATION DEPENDS UPON NERVE GROWTH FACTOR PRODUCED BY LEPTIN RECEPTOR+ STROMAL CELLS AND ADIPOCYTES

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Leptin Receptor-expressing (LepR+) stromal cells in adult bone marrow are highly enriched for growth factors required for the maintenance of hematopoietic stem cells, including SCF, CXCL12, and Pleiotrophin. Nerve fibers in the bone marrow also regulate important aspects of hematopoiesis, particularly hematopoietic regeneration; however, it remains unknown what cells in the bone marrow synapse with nerve fibers or what target cells provide the neurotrophic factors required by nerve fibers. Here we discovered that LepR+ cells are the main source of Nerve Growth Factor (NGF) in normal young adult bone marrow. Deletion of Ngf in LepR+ cells eliminated bone marrow nerve fibers, including both sympathetic and sensory nerves. After myeloablation, adipocytes expand dramatically in number in the bone marrow and become the main source of some growth factors including SCF, which is required for hematopoietic regeneration. Adipocytes also express NGF. After myeloablation, the level of bone marrow NGF increased, promoting more extensive bone marrow innervation. Deletion of Ngf from LepR+ cells led to the loss of NGF from both the LepR+ cells themselves as well as the adipocytes they formed after myeloablation, delaying hematopoietic regeneration. This was phenocopied by sympathetic nerve ablation using 6-hydroxydopamine. Sympathetic nerves promoted hematopoietic regeneration partly by promoting SCF expression by adipocytes as a consequence of beta-adrenergic receptor activation on adipocytes. Bone marrow denervation with Ngf deletion, pharmacological ablation of sympathetic nerves, or deletion of beta-adrenergic receptors all reduced Scf expression by adipocytes and delayed hematopoietic regeneration. The beta2-adrenergic receptor agonist salbutamol promoted Scf expression by adipocytes in denervated bone marrow, rescuing hematopoietic regeneration. Nerve fibers thus depend upon trophic factors produced by LepR+ stromal cells and adipocytes in the bone marrow and regulate hematopoietic regeneration partly by promoting SCF expression by adipocytes.

Keywords: Bone Marrow Niche, Hematopoietic Regeneration, Nerve Growth Factor

Poster: 157

A COMPREHENSIVE CHARACTERIZATION OF BIODISTRIBUTION AND SINGLE-CELL STATES OF HSC-DERIVED MICROGLIA UPON EX-VIVO LENTIVIRAL GENE THERAPY IN MURINE MODELS OF NEUROLOGICAL DISEASE

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The genetic modification and administration of hematopoietic stem cells (HSCs) is an established cell and gene therapy platform for the treatment of monogenic disorders. Clinical trials using ex vivo genetic modification and infusion of autologous HSCs have successfully addressed diseases with hematopoietic, peripheral, and central nervous system (CNS) involvement. Here we present a high throughput biodistribution study of genetically engineered mouse HSCs upon two routes of administration (intravenous [IV]; and intracerebroventricular [ICV]) in busulfan-conditioned recipient mice. We then tested the ability of our platform to induce expression of therapeutically relevant levels of transgene in two mouse models of neurodegeneration. We show that IV-administered HSCs engraft widely in all murine tissues including the CNS. ICV-administration results in chimerism limited only to the CNS. In contrast with previous reports, IV-administration showed increased total CNS engraftment over ICV administration, a difference confirmed across multiple related endpoints. We observed that the majority of CNS-engrafted cells express canonical markers of microglia and have highly ramified morphology consistent with the surveilling role of endogenous microglia. We FACS-sorted and analyzed endogenous and HSC-derived microglia from the brain via single cell RNA-Seq at 13 months post-dose. By profiling 29,085 single cell transcriptomes, we established that CNS-engrafted HSC-derived microglia express bone fide microglial markers and exhibit a transcriptional profile similar to endogenous microglia. Strikingly, we identified a set of distinct transcriptional states and key markers (e.g. Apoe and Mrc1) that discriminate engrafted microglia versus endogenous. We then treated GbaD409V Parkinson's disease and GRNR493X FTD mouse models with cells expressing human GBA and GRN respectively. We observed stable supraphysiological expression of transgene in the periphery and the CNS in both models. Our findings shed new light on the characteristics of HSC-derived microglia and support the application of ex vivo lentiviral HSC gene therapy as a platform by which a single systemic administration of genetic payloads may address both systemic and neurological pathobiology.

Keywords: microglia, gene therapy, hematopoietic stem cells

Poster: 158

CIRCADIAN REGULATION OF MOUSE MYOGENIC DIFFERENTIATION AND MUSCLE REGENERATION VIA IGF2 ACTIVATION

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Circadian rhythms regulate cell proliferation and differentiation but circadian control of tissue regeneration remains elusive at the molecular level. Here, we show that the circadian master regulators Per1 and Per2 are integral components defining the efficiency of myoblast differentiation and muscle regeneration. We found that the depletion of Per1 or Per2 suppressed myoblast differentiation in vitro and muscle regeneration in vivo, demonstrating their non-redundant functions. Both Per1 and Per2 directly activated Igf2, an autocrine promoter of myoblast differentiation, accompanied by Per-dependent recruitment of RNA polymerase II, dynamic histone modifications at the Igf2 promoter and enhancer, and the promoter-enhancer interaction. This circadian epigenetic priming created a preferred time window for initiating myoblast differentiation. Consistently, muscle regeneration was faster if initiated at night when Per1, Per2, and Igf2 were highly expressed compared with morning. This study reveals the circadian timing as a significant factor for effective muscle cell differentiation and regeneration.

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Keywords: myogenesis, muscle regeneration, circadian rhythms

Poster: 159

SINGLE CELL DECONSTRUCTION OF MURINE VOLUMETRIC MUSCLE LOSS REVEALS NATURAL KILLER CELL AND NEUTROPHIL IMBALANCES PREVENT MUSCLE STEM CELL MEDIATED REGENERATION

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Volumetric muscle loss (VML) overwhelms the innate regenerative capacity of mammalian skeletal muscle, leading to numerous disabilities and reduced quality of life. Immune cells are critical responders to muscle injury and guide muscle stem cell (MuSC) mediated myogenic repair. However, how immune cell infiltration and inter-cellular communication networks are altered following VML to drive pathological outcomes remains unclear. Thus, we sought to characterize the cellular and molecular mechanisms driving fibrotic degeneration of skeletal muscle after VML by comparing the healing trajectories between

muscle loss injuries that regenerate to those that result in fibrosis. Using single-cell RNA sequencing (scRNA-Seq), lineage-tracing mouse models, in vitro assays, histological and functional analysis, cell transplants, and in vivo small molecule inhibition, we elucidate new cellular and molecular players post VML. We observed that degenerative VML injuries result in persistent infiltration of inflammatory neutrophils. Cytolytic natural killer (NK) cells were also observed to accumulate in degenerative defects and interact with neutrophils via secretion of chemokine receptor type 1 (CCR1) ligands. Intramuscular NK cell transplants significantly reduced neutrophil abundance and enhanced healing rates, while systemic delivery of a CCR1 inhibitor exacerbated neutrophil accumulation. As a consequence of exacerbated neutrophils in degenerative injuries, we observed impairments in MuSC myogenic capacity. The reductions in regenerative potential of MuSCs were also impacted by over-expression of transforming growth factor beta 1 (TGFb1). Local inhibition of TGFb1 signaling improved tissue morphology and maximal tetanic force, and lowered neutrophil populations. Together, these findings enhance our understanding of immune cell-stem cell communication dynamics governing muscle healing outcomes. This work provides a valuable resource for further exploration into mechanisms driving VML-induced fibrosis and may help elucidate drivers of fibrosis and chronic inflammation in other pathologies.

Keywords: stem cell niche, skeletal muscle, regeneration

Poster: 160

EXCITATORY AMINO ACID TRANSPORTER 1 (EAAT1) IS NECESSARY FOR GLUTAMATE-STIMULATED PROLIFERATION OF ADULT MOUSE HIPPOCAMPAL NEURAL STEM CELLS

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Adult neurogenesis supports hippocampal cognition and responds dynamically to physiological and cognitive experience. Activity-driven signaling by the neurotransmitter glutamate is a potential mechanism by which experiences regulate the neurogenic cascade. However, while glutamate is well known to stimulate neurogenesis, remarkably little is known about the molecular mediators. We aimed to identify these molecular mediators in adult hippocampal radial neural stem cells (NSCs). Using a well-characterized monolayer culture model of adult hippocampal NSCs, we found that pharmacological inhibition of excitatory amino acid transporter (EAAT)-mediated glutamate transport, but not of ionotropic and metabotropic glutamate receptors, impaired proliferation and prevented the pro-proliferative effect of glutamate. We then investigated which EAATs mediate glutamate transport in NSCs and found almost exclusive dependence on EAAT1. Similarly, specific pharmacological inhibition of EAAT1 reduced proliferation and prevented the pro-proliferative effects of glutamate. Next, we used bulk RNA seq and gene ontology analysis to interpret the biological processes that differ between NSCs treated with glutamate and EAAT inhibitors. We found that separately, glutamate induced a network supporting macromolecule biosynthesis while EAAT inhibition induced processes related to cell migration and downregulated processes related to cell cycle progression and mitosis. Furthermore, the majority of genes regulated by glutamate alone were regulated in the opposite direction by the combination of glutamate and EAAT inhibition, which suggests that EAAT inhibition largely suppresses the transcriptional program of glutamate. Together, our data indicate that EAAT1-dependent glutamate transport is necessary for glutamate-stimulated proliferation of hippocampal NSCs. Ongoing studies are investigating the in vivo consequences of glutamate transport for adult neurogenesis. Overall, we expect these studies to improve our understanding of how neurotransmitter signaling impacts neurogenesis in the mature brain, and possibly reveal opportunities for regenerative strategies.

Keywords: Neurogenesis, Glutamate, Neural stem cell

Poster: 161

PLG BIOMATERIAL BRIDGE IMPLANTATION AND HUMAN NEURAL STEM CELL TRANSPLANTATION FOR THE REPAIR OF SPINAL CORD INJURY

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Spinal cord injury (SCI) causes a disruption of the blood-spinal cord barrier, which allows the infiltration of immune cells and components from the blood to surround the spinal cord tissue. This leads to further degeneration of spinal cord tissue including, neuronal and glial cell death, axonal loss, demyelination and the formation of an adverse inhibitory environment that prevents regeneration. In order to foster functional regeneration and reconstitute the injured spinal cord, we studied a combinatorial approach based on two therapeutic interventions: (I) implantation of a biomaterial bridge comprised of a biodegradable copolymer, poly (lactide-co-glycolide) (PLG) bridge, and (II) transplantation of human neural stem cells (hNSC). Acute PLG-bridge implantation stabilizes the injury site and fosters the growth of sensory and motor axons, including fibres of the corticospinal tract. However, analyses of axonal growth within the PLG-bridge demonstrate that a small percentage of regenerated axons become myelinated, an essential factor for efficient transmission of descending signals from the brain. We have previously shown that delayed hNSC transplantation in a mouse SCI model results in high levels of human oligodendroglial differentiation and remyelination. We therefore hypothesized that the combination of bridge implantation with hNSC transplantation could enhance functional regeneration and locomotor recovery following SCI. In parallel, we established an in vitro model to test the effect of exposure of hNSC to a 3-D PLG-bridge on oligodendroglial fate selection in the presence or absence of inhibitory immune cues. Collectively, our data suggest that the combination of PLG bridge implantation and hNSC transplantation supports axon regeneration and oligodendroglial fate selection.

Keywords: Spinal Cord Injury, Neural stem cells, Biomaterial

Poster: 162

NEURAL REGENERATION IN ADULT DROSOPHILA

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Adult neurogenesis occurs at variable extents in specific locations of the vertebrate nervous system throughout life, and its deregulation has been linked to diverse pathologies. Therefore, interventions aimed at promoting functional neurogenesis upon injury and ageing represent a promising strategy to rejuvenate the adult nervous system. With an unparalleled genetic and functional toolbox, Drosophila represents a remarkable model to accelerate the dissection of mechanisms promoting neural regeneration. Yet, generation of new neurons in the adult fly has been elusive thus far. Here I present evidence for generation of new neurons in the optic lobes, the auditory/vestibular, as well as the olfactory system of adult flies. Furthermore, acute injury and oral administration of small molecules enhance neuronal regeneration. We generated a modified lineage tracing system that allows for the sustained identification of adult-born neurons in the fly and their genetic manipulation in a cell type-specific manner. These results unveil the hidden proliferative capacity in the adult Drosophila nervous system, and underscore its compelling potential to expedite the identification of conserved mechanisms and compounds promoting nervous system regeneration at the single-cell, circuitry, and functional levels.

Funding Source: USC-CONACYT Postdoctoral Fellowship; USC Provost's Postdoctoral Research Grant and Undergraduate Fellowship; NIH (R00NS089013, R56AG064077), Whittier Foundation, Baxter Foundation and Broad Foundation

Keywords: Adult neural regeneration, Small molecules, Drosophila

Poster: 163

TARGETING MITOCHONDRIA TO ENHANCE HUMAN NEURAL STEM CELL FUNCTION FOR NEUROTRANSPLANTATION

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Transplantation of human neural stem cells (hNSC) to repair damaged tissues in traumatic injuries has shown promise in pre-clinical studies and early clinical trials. Spinal cord injury (SCI) is marked by a secondary injury phase characterized by an ischemic oxidative microenvironment, secreted pro-inflammatory factors, and mitochondria dysfunction in the surviving damaged cells/tissues. We hypothesize that survival and engraftment of transplanted hNSCs is hindered in a manner reliant on their "mitochondria fitness traits" (MFTs). Mitochondria are vital for providing the energy (bioenergetics) of cells during times of high demand such as during proliferation, stem cell differentiation and migration. To test this hypothesis, we utilize tissue-derived hNSC lines derived in our lab, previously tested for their repair potential (efficacy) in restoring functional recovery after SCI. Transcriptomic analysis comparing an efficacious line (UCI161) versus a non-efficacious line (UCI152) revealed differences in key MTFs: mitochondrial bioenergetics, biogenesis, permeability transition/redox potential, and autophagy/mitophagy. We identified drug candidates to target mitochondrial function from by screening FDA-approved mitochondrial drugs, testing their effects on hNSCs after 48h of treatment. Treatment of hNSC with a bioenergetics-enhancing drug led to increased mitochondrial hyperfusion and tunneling nanotube (TNT) formation, whereas treatment of hNSC with a biogenesis drug led to enhanced mitochondria mass and proliferation in UCI161. MTT, autophagy analysis, mitochondria membrane potential (MMP) and ATP analysis revealed UCI161 were better responders to bioenergetic drugs, where UCI152 responded optimally to biogenesis enhancement. hNSC with improved mitochondrial response capabilities show promise for translation to neuro-transplantation in chronic traumatic injuries characterized by mitochondria dysfunction and loss of bioenergetics.

Funding Source: Office of UC President's Postdoctoral Fellowship

Keywords: Neural Stem Cell Transplantation, Mitochondria, Spinal Cord Injury

Poster: 164

IN VITRO AND IN VIVO CHARACTERIZATION OF CRISPR-CAS9 EDITED HUMAN PLACENTAL ADHERENT STROMAL CELLS WITH LOW TISSUE FACTOR ACTIVITY

Huang, Chenfei[1], Ye, Qian[1], Gleason, Joseph[1], Shah, Navjot[1], Rousseva, Valentina[1], Stout, Bhavani[1], He, Shuyang[1], Hariri, Robert[2], Zhang, Xiaokui[1]

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Human placental adherent stromal cells (ASCs) are culture-expanded, undifferentiated MSC-like cells derived from full-term postpartum placenta tissue and are shown to possess immunomodulatory and pro-angiogenic activities in vitro and in vivo (He et al, 2017). Since ASCs express tissue factor (TF), an activator of coagulation, we propose that TF knockout (TFKO) by CRISPR gene editing will improve the safety features of these cells. Here, we report the characterization of phenotype, proliferation, and immunomodulatory properties of ASCs-TFKO in development of a next-generation cell therapy candidate. ASCs derived from four different donors were transfected with sgRNAs targeting TF gene and Cas9 protein and expanded for 10-16 accumulated population doublings. Non-edited ASCs cultured by the same procedures were used as control (CT). In ASCs-TFKO cells, TF gene editing efficiency was 91.0% ± 7.6% determined by the TIDE assay and the expression on cell surface was reduced by 97.6% ± 1.2% by FACS comparing with CT. The TF activity of ASCs-TFKO was significantly reduced to 12.2% ± 8.2% of CT ($p<0.05$). The immunophenotype of ASCs-TFKO (CD34-CD10+CD105+CD200+) was not affected by gene editing. During culture expansion, ASCs-TFKO showed similar growth capacity as CT. The secretome profiles of over 40 cytokines, chemokines and growth factors were comparable between ASCs-TFKO and CT. In response to IL-1 stimulation, ASCs-TFKO secreted the similar range (2000-6000 pg/mL) of immunomodulatory molecule PGE2 as compared with CT. When cocultured with CD4+ and CD8+ T cells activated by anti-CD3/CD28 beads, ASCs-TFKO significantly suppressed the proliferation of activated T

cells ($p<0.05$). Furthermore, intravenous administration of ASCs-TFKO significantly reduced disease score and weight loss in an EAE mouse model ($p<0.0001$), suggesting TFKO did not affect the immunomodulatory activities of ASCs cells. In summary, we have established an effective TFKO method in ASCs by CRISPR-Cas9 and demonstrated > 90% of efficiency at both gene and protein levels. ASCs-TFKO showed low TF activity and retained MSC-like cell phenotype, proliferation capability, in vitro and in vivo immunomodulatory functions of the non-edited ASCs. These data warrant the further development of ASCs-TFKO as a novel candidate for cell therapy.

Keywords: Placenta-derived MSC-like adherent cells, CRISPR gene editing, immunomodulation

00:00 - 1:00 EDT

POSTER SESSION 2

NEW TECHNOLOGIES

Poster: 201

RCOR2 IS A COMPONENT OF THE PLURIPOTENCY REGULATORY NETWORK AND IS REQUIRED FOR EDDICIENT ESC DIFFERENTIATION

Larcombe, Michael R.[1], Knaupp, Anja[1], Mohenska, Monika[1], Ford, Ethan[2], Lim, Sue Mei[1], Wong, Kayla[1], Chen, Joseph[1], Firas, Jaber[1], Huang, Cheng[3], Liu, Xiaodong[1], Nguyen, Trung[2], Sun, Yu Bo Y.[1], Holmes, Melissa[1], Tripathi, Pratibha[1], Pflueger, Jahnvi[2], Rossello, Fernando[1], Schroder, Jan[1], Davidson, Kathryn[1], Das, Partha[1], Nefzger, Christian[1], Lister, Ryan[2], Haigh, Jody[1], Schittenhelm, Ralf[3], Polo, Jose[1]

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Embryonic stem cells are pluripotent and can therefore be expanded indefinitely while retaining full developmental potential and proliferative capacity. ESCs resist differentiation and sustain self-renewal through maintenance of the pluripotency gene regulatory network. This network is centered around the key transcription factors (TFs) OCT4, SOX2 and NANOG, operating in a highly dynamic and combinatorial manner to retain pluripotency gene expression whilst suppressing lineage-specific genes. These master regulators are tightly regulated to ensure ESCs can rapidly respond to developmental cues, however, how this occurs is not fully understood. Using our single locus isolation method (TINC) we identified the corepressor RCOR2 as a direct regulator of the core pluripotency factors and demonstrated extensive binding to genes enriched for various developmental processes. To further investigate the role of RCOR2, we generated CRISPR Cas9 knockout ESC lines. Deletion of Rcor2 resulted in a loss of the typical dome-shaped morphology, with cells appearing differentiated. Yet Rcor2 -/- ESCs retained pluripotency potential, giving rise to cells from all three germ layers in teratoma assays. Furthermore, RNA-sequencing revealed Rcor2 -/- ESCs exhibit a naive pluripotency transcriptional signature with upregulated expression of Nanog and Oct4. In order to examine their ability to exit the pluripotency program, we subjected the Rcor2 -/- ESCs to an embryoid differentiation assay. Interestingly, they formed irregular embryoid bodies that failed to repress genes critical for pluripotency and failed to upregulate genes involved in primitive streak formation. Together, these findings indicate that the corepressor RCOR2 is a component of the pluripotency regulatory network and is required for efficient exit from the pluripotent state.

Keywords: chromatin, epigenetics, Nanog

Poster: 202

NOVEL TECHNOLOGIES ON AVIAN STEM CELL ENGINEERING

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Pluripotent stem cells were isolated from the early avian embryos (Stage X; blastoderm). Molecular characterization of the stem cell development and differentiation was analyzed. Major regulator genes on avian stem cells; Cvh, Nanog, Tert, were analyzed. Insertion of the regulatory genes could be conducted into the donor stem cells prior to injection into the recipients. Genome editing has been conducted for germline chimeras. Functional leg or heart could be regenerated. Germline chimera brought the novel strategies to poultry breeding. Novel technologies on avian stem cell engineering could elucidate the basic mechanism of avian development and genetic conservation.

Keywords: stem cell, chimera, avian

Poster: 203

DISSECTION OF THE NANOG REGULATORY COMPLEX AT SINGLE LOCUS RESOLUTION REVEALS EPIGENETIC MECHANISMS FOR PLURIPOTENCY MAINTENANCE AND EXIT

Mohenska, Monika[1], Knaupp, Anja[1], Larcombe, Michael[1], Ford, Ethan[2], Lim, Sue Mei[1], Wong, Kayla[1], Chen, Joseph[1], Firas, Jaber[1], Huang, Cheng[3], Liu, Xiaodong[1], Nguyen, Trung[2], Sun, Yu Bo Yang[1], Holmes, Melissa[1], Tripathi, Pratibha[1], Pflueger, Janhvi[2], Rossello, Fernando[1], Schroder, Jan[1], Davidson, Kathryn[1], Nefzger, Christian[1], Das, Partha[1], Haigh, Jody[4], Lister, Ryan[2], Schittenhelm, Ralf[3], Polo, Jose[1]

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Determination of cellular fate is achieved by the interaction between proteins and regulatory elements (REs) to control gene expression. Although many advances in epigenome profiling have broadened our understanding of RE utilization, it has been challenging to identify factors that interact with REs due to a lack of appropriate techniques. Hence, we developed TINC: TALE-mediated isolation of nuclear chromatin. Furthermore, we optimized a single locus specific bioinformatics pipeline which can be utilized for improved analyses of this nature. We applied TINC to dissect the protein complex at the Nanog promoter in embryonic stem cells (ESCs) and identified a myriad of regulators, including transcriptional activators, histone modifiers and repressors. One of these proteins was the corepressor RCOR2, which we further validated to be an integral regulator of the core pluripotency network. For example, RCOR2 was found to share many genomic targets with the pluripotency master regulators OCT4 SOX2 and NANOG and plays a role in fine-tuning the expression of most active pluripotency genes. Additionally, our data suggest that RCOR2 functions as part of the NuRD complex and besides pluripotency maintenance plays a major role in silencing the pluripotency network during differentiation. Altogether, our findings led us to further define regulatory

mechanisms of the epigenetic landscape and suggest that a highly complex and coordinated interplay of numerous factors is required for correct NANOG levels for ESC self-renewal as well as on-demand differentiation progression.

Keywords: single-locus, Nanog, bioinformatics

Poster: 204

OSCILLATED MECHANICAL STIMULATION FACILITATES FUNCTIONAL DIFFERENTIATION OF HEPATOBLASTS

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Human hepatoblasts (hHBs) play an important role in liver development in vivo and in organoid engineering in vitro. Conventional differentiation from human pluripotent stem cells (hPSCs) to hHBs generally uses chemical factors, such as fibroblast growth factor (FGF) and bone morphogenetic protein (BMP). However, since these protocols are insufficient to obtain functional hHBs, additional factors for HB differentiation protocols need to be identified to improve hHB functionality. Here, we hypothesized that mechanical stimulation by heart beating might facilitate the differentiation from endoderm to hHBs, because in vivo hHBs are located next to the heart and might be influenced by its beating. To confirm our hypothesis, we developed a microfluidic device made of biocompatible and stretchable polydimethylsiloxane, enabling oscillating mechanical stimulation to mimic heart beating onto cultured cells. On the device coated with Matrigel extracellular matrices, hPSCs were differentiated into endoderm cells with activin A, BMP4, FGF2, and LY294002. The oscillated mechanical stimulation in addition to chemical factors (FGF and BMP) was applied to the endoderm cells for 3 d to induce hHBs. To investigate the effect of oscillatory mechanical stimulation on the functionality of hHBs, the activities of cytochrome P450 3A (CYP3A) hepatic metabolic enzymes were measured. Stimulated hHBs showed significantly higher CYP3A activity than unstimulated hHBs. Moreover, by performing immunocytochemistry, we confirmed that stimulated hHBs expressed HB functional proteins, such as albumin, CYP3A7, and cytokeratin 19 proteins, more than those of unstimulated hHBs. In addition, hepatocyte-like cells (HLCs) were differentiated from hHBs treated with hepatocyte growth factor and oncostatin M after stimulation was stopped. HLCs derived from stimulated hHBs showed two-fold higher CYP3A activity than HLCs derived from unstimulated hHBs. In summary, oscillatory mechanical stimulation facilitates the functional differentiation of hHBs as well as HLCs in vitro. Therefore, mechanical stimulation is one of the most important factors in liver development.

Funding Source: the Japan Society for the Promotion of Science Japan Agency for Medical Research and Development

Keywords: Microfluidic device, Mechanical stimulation, Hepatic differentiation

Poster: 205

CORRELATION BETWEEN CELL MORPHOLOGICAL INFORMATION OBTAINED FROM DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY (D-IHM) AND CANCER STEM CELL-LIKE CHARACTERISTICS OF HUMAN COLON CANCER

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D-IHM images provide cell morphology information quantitatively by non-invasively measuring the phase delay of cultured cells. We have previously shown the utility of cell-specific scores obtained from D-IHM to predict the cell function in mesenchymal stem cells and epithelial cells. This study will report on the utility of morphological indicators derived from D-IHM as a novel method for predicting cancer stem cell (CSC)-like characteristics of colon cancer cells (CAC). CSCs have strong tumorigenicity and are closely related to anti-cancer drugs' resistance, tumor recurrence and metastasis. Colon CSCs have been known to express specific biomarkers, such as CD133, hypoxia-inducible factor (HIF)-1 and Cdx2. CD133 promotes the growth of cancer cells by activating the Wnt signaling pathway. HIF-1 promotes the dedifferentiation of cancer cells in the hypoxic environment and enhances stem cell properties. Cdx2, a homeobox transcription factor involved in the differentiation and maintenance of intestinal epithelial cells, suppresses malignant transformation of CAC and the stemness of CSC. They are usually evaluated by quantitative PCR or flow cytometry cell-invasively. Alternatively, the sensitivity of CAC to anti-cancer drugs has been evaluated by the survival rate of cancer tissue-derived cells represented by the culture drug sensitivity test and histoculture drug response assay. Recently, genomic cancer medicine has been widespread, whereby gene mutations are comprehensively analyzed and optimal anti-cancer drugs are selected for each patient. However, this test is available only to patients with limited conditions. Therefore, since conventional chemotherapy is still the first choice for most patients, assessing the drug susceptibility concerning CSC-like characteristics has great significance in providing effective treatment. Alternatively, we found that the survival rate and expression of some biomarkers, representing CSC-like characteristics, have not necessarily correlated in the 5-FU addition experiment for CAC. Therefore, we focused on D-IHM images, which provided two cell morphological indicators, each associated with CAC-like characteristics and cell proliferation. We will report on their relevance including the association with cell cycle and apoptosis.

Funding Source: Shimadzu Corporation

Keywords: Digital in-line holographic microscopy, Cancer stem cell, Colon cancer

Poster: 206

SINGLE-CELL CLONING OF HUMAN INDUCED PLURIPOTENT STEM CELLS: AUTOMATION AND PROTOCOL OPTIMIZATION

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CiRA Foundation's Facility for iPS Cell Therapy is a Cell Processing Center responsible for manufacturing of clinical-grade iPS cells, and our newest project is generation of HLA-genome-edited iPS cell lines, designed to reduce the risk of immune rejection, by targeting HLA-A, HLA-B and CIITA genes for CRISPR/Cas9-based knock-out. One of the challenges in the manufacturing of genetically engineered iPS cells is single-cell cloning, specifically, choice of the optimal cell dispensing technology, confirmation of clonality, dependable imaging technique and assuring survival of single iPS cell in culture. Here we report our progress in development of the single-cell cloning process suitable for manufacturing of clinical-grade iPS cells with adherence to cGMP regulations. First, we evaluate several single-cell dispensing instruments – CellCelector™ (ALS Automated Lab Solutions), f.sight and UP.SIGHT (Cellink/Cytena) and VIPS™ (Solentim) – with regard to reliability of single cells identification and monoclonality verification. Next, we compare the technologies based on dispensing single cells into individual wells of the multiwell plate with systems utilizing nanowell arrays for single-cell isolation and clonal expansion. Finally, we are optimizing culture protocols, specifically, comparing various supplements to ensure single cell survival:Y-27632 ROCK inhibitor, CEPT cocktail (NCATS), CloneR reagent (STEMCELL Technologies) and RevitaCell supplement (Invitrogen), choosing plate coating method for optimal cell attachment and identifying culture plate type for highest imaging quality.

Funding Source: This research was supported by Japan Agency for Medical Research and Development (AMED) under Grant Number JP20bm0104001.

Keywords: single-cell cloning, automation, iPSC

Poster: 207

THE EFFECT OF FUCOIDAN ON 3D-PRINTED FULL-THICKNESS HUMAN SKIN FOR IMPROVING LONG-TERM STABILITY

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Many researchers have been devoted to the development of a full-thickness biomimetic human skin equivalent (HSE) in vitro. However, a full-thickness biomimetic HSE in vitro is complicated by limited understanding of cross-bridge in the multi-cellular cooperation of keratinocyte, fibroblasts etc., multi-layered complexity of human skin, long-term stability, and vitality of HSE during long-term culture. We investigated whether fucoidan, a complex polysaccharide derived from brown seaweed, enable the long-term maintenance of full-thickness of HSE using 3D bioprinted-dermal scaffolds. 3D-bioprinted dermal scaffolds fabricated with a 3DX bioprinter and extrusion-based bioprinting method with collagen/fibroblast bioink. After 1 days, HaCaT human immortalized keratinocytes were seeded onto the 3D bioprinted-dermal scaffold-on-frame constructions and cultured in the presence and/or absence of fucoidan with airlifting condition for 7 to 28 days. Western blot analysis and immunohistochemistry staining was used to express the function of a full-thickness HSE in the presence and/or fucoidan. The results show that fucoidan could improve the maintenance and survival rate of a full-thickness HSC more than control. Autophagy has been contributed to response of keratinocytes to skin aging. In agreement with these observations, fucoidan was associated with the expression of autophagy-related biomarkers in keratinocytes under differentiation condition. Our findings provide insight into the application of fucoidan for improving long-term stability the 3D architecture of full-thickness biomimetic HSE in vitro.

Funding Source: 2017R1A6A03015562, and 2020R1I1A1A01054595 of the National Research Foundation funded by the Korea government.

Keywords: 3DX printer, fucoidan, full-thickness SKIN

Poster: 208

ENGINEERED CAR-MACROPHAGES FROM HUMAN PLURIPOTENT STEM CELLS FOR CANCER IMMUNE CELL THERAPIES

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The Chimera antigen receptor (CAR)-T cell therapy has gained great success in the clinic. However, there are still major challenges for its wider applications in a variety of cancer types including lack of effectiveness due to the highly complex tumor microenvironment, and the forbiddingly high cost due to the personalized manufacturing procedures. In order to overcome these hurdles, numerous efforts have been spent focusing on optimizing Chimera Antigen Receptors, engineering and improving T cell capacity, exploiting features of subsets of T cell or NK cells, or making off-the-shelf universal cells. Here, we developed induced pluripotent stem cells (iPSCs)-derived, CAR-expressing macrophage cells (CAR-iMac). CAR expression confers antigen-dependent macrophage functions such as expression and secretion of cytokines, polarization toward the pro-inflammatory/anti-tumor state, enhanced phagocytosis of tumor cells, and in vivo anti-cancer cell activity. We also genetically engineered a series of payloads to enhance CAR-iMac's controled polarization, chemotraction and to overcome checkpoint. This technology platform provides an unlimited source of iPSC-derived engineered CAR-macrophage cells which could be utilized to eliminate cancer cells.

Keywords: iPSC derived CAR-macrophages, Cancer immunotherapy, Bioengineering

Poster: 209

CHEMICALLY-DEFINED AND FEEDER-FREE MAINTENANCE OF CANINE INDUCED PLURIPOTENT STEM CELLS

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Canine induced pluripotent stem cells (ciPSCs) demonstrate potential for use in veterinary regenerative medicine, genetic disease modeling, and drug screening. Traditionally, ciPSC culture maintenance has required feeder cells (such as murine embryonic fibroblasts (MEFs)) and medium containing fetal bovine serum (FBS) or KnockOut Serum Replacement (KSR). However, MEF, FBS, and KSR inherently provide chemically undefined components originating from other organisms, which increases the risk of immune-mediated rejection following ciPSC transplantation. Moreover, the traditional ciPSC generation method incorporates mechanical passage, which is a skilled technique requiring significant time and effort. Such challenges represent obstacles to routine clinical application of ciPSCs. The present study demonstrated that a combination of StemFit® AK02N and iMatrix-511 was sufficient for successful culture of ciPSCs previously maintained using conventional methods. Moreover, footprint-free ciPSCs generated de novo from canine peripheral blood mononuclear cells using N2B27 and StemFit® AK02N could also be successfully maintained using StemFit® AK02N and iMatrix-511, and were readily expandable using the enzymatic single-cell passage method. Such ciPSCs exhibited normal karyotypes, expressed multiple pluripotency markers (including OCT3/4, NANOG, and SSEA1), and were able to differentiate into all three germ layers in vitro (as indicated by expression of the endoderm marker SOX17, the mesoderm marker DESMIN, and the ectoderm marker TUBB3). Moreover, ciPSCs also demonstrated the ability to form teratomas incorporating all three germ layers. Taken together, findings demonstrate that ciPSCs can be maintained under chemically-defined and feeder-free conditions, and are readily expandable using StemFit® AK02N and iMatrix-511. In conclusion, the method presented herein possesses several advantages which may contribute to facilitating routine use of ciPSCs in both pathogenesis-elucidating preclinical research and regenerative veterinary medicine.

Funding Source: This work was supported by Japan Society for the Promotion of Science, Grant/Award Numbers JP18K19273, JP18H02349, and JP19J22851 and The Japan Science Society, Sasakawa Scientific Research Grants, Grant/Award Number 2020-4079.

Keywords: Canine Induced Pluripotent Stem Cells, Feeder-free, chemically-defined

Poster: 210

AGGREGATION-INDUCED EMISSION (AIE) NANOPARTICLES LABELED HUMAN EMBRYONIC STEM CELLS (HESCS)-DERIVED NEURONS FOR BRAIN TRANSPLANTATION

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Intracerebral transplantation of neurons derived from stem cells has emerged as a potential therapeutic strategy to halt or to reverse the progression of neurodegenerative diseases such as Parkinson's disease (PD). One important aspect of transplantation is the accessibility to track and control the activity of the stem cells-derived neurons post-transplantation. Current imaging modalities used to visualize transplanted grafts include MRI, SPECT, and PET imaging. However, patients would have to undergo multimodular imaging procedures to examine graft survival, innervation into host tissues, and to access functional DA release. In efforts to improve the spatiotemporal resolution of functional neuroimaging of transplanted neuronal grafts, here, we have synthesized organic nanoparticles (NPs) with aggregation-induced emission (AIE) characteristics that would allow for efficient external cell labeling of human embryonic stem cells (hESCs)-derived neurons. In addition, we have demonstrated the biocompatibility of AIE-NPs in cultured mouse neuronal progenitor cells (NPCs) and hESC-derived neurons both showing high degree of intracellular penetration and long-term retention in vitro without altering the neuronal proliferation, differentiation, and viability. Furthermore, we have traced AIE-NPs labeled neuronal grafts in mouse brain striatum in various time points post-transplantation and have observed prolonged cellular retention of AIE-NPs labeled neuronal grafts up to 1 month post-transplantation with relatively low cellular toxicity. Lastly, we have shown activation of brain microglia in response to AIE-NPs labeled grafts. Together, these findings highlight the potential application of AIE-NPs in neuronal transplantation and to improve the spatiotemporal resolution of functional neuroimaging in hopes to better refine the accuracy and quality of post-operative care.

Funding Source: SingHealth Fund Limited (SHF-Foundation) (SHF/FG658P/2017), Singapore National Research Foundation (R279-000-483-281) and, Parkinson's disease Translation Clinical Research grant.

Keywords: Neuronal Transplantation, aggregation-induced emission nanoparticles, Biomarker

Poster: 211

DEVELOPMENT OF A NEW GENOME EDITING SYSTEM AND APPLICATION TO GLIOBLASTOMA TREATMENT

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Glioblastoma (GBM), a primary malignant brain tumor in adult, which is characterized by proliferation due to glioma stem cell (GSC), shows a strong resistance to conventional therapy. A variety of tumor cells are reported to show pathologically enhanced uptake of glucose and anaerobic glycolysis, which is called Warburg's effect. Hexokinase (HK) is overexpressed in various types of malignant tumors, and is a kinase which phosphorylates glucose and produces glucose-6-phosphate (G6P), which is essential for initiating nucleic acid synthesis pathway, pentose phosphate pathway (PPP). Even though previous studies reported that inhibition of the enzymatic activity of HK2, a HK isozyme, could significantly lower proliferation of GBM, there are some obstacles in its clinical application: HK isozymes are expressed in different type of cells and vital for cell survival. Since conventional such method to lower HK activity as gene knockout or knockdown, lack cell type specificity, it is a challenge to block GBM growth without affecting normal cells in vivo by targeting HK family. In order to provide an ideal solution to this problem and find an effective GBM treatment, we focused on glycolytic metabolism of GBM, using a newly designed genome editing method which enables GBM specific HK knockout. In this study, we found a new candidate gene for GBM's marker that has been reported to have a critical role in cell growth and is a potential target for the new method of genome editing, according to multivariate logistic regression analysis using transcriptome data on patient derived GBM cells. We also revealed that HK families are key enzymes to produce G6P in GBM cells compared with other enzymes and the expression patterns of HKs are different between GBM and GSC by utilizing RNA seq data of genes that are related to PPP. Thus, gene knockdown on multiple HK subtypes could effectively prevent GBM growth. Based on these findings, we are currently establishing the GBM specific gene knockout method and apply it to HKs.

Keywords: Glioblastoma multiforme, Transcriptome analysis, Genome editing

Poster: 212

REAL-TIME MONITORING OF GLUTATHIONE DYNAMICS DEMONSTRATES THE FUNCTIONAL ROLES OF CREB1-NRF2 PATHWAY FOR MESENCHYMAL STEM CELLS THERAPEUTIC POTENCY

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Glutathione (GSH), the most abundant non-protein thiol functioning as an antioxidant, plays critical roles in maintaining the core functions of mesenchymal stem cells (MSCs), which are used as a cellular immunotherapy for graft-versus-host disease (GVHD). However, the significance of GSH dynamics in MSCs remains elusive. Genome-wide gene-expression profiling and high-throughput live-cell imaging assays revealed that CREB1 enforced the GSH-recovering capacity (GRC) of MSCs through NRF2 by directly up-regulating NRF2 target genes responsible for GSH synthesis and redox cycling. MSCs with enhanced GSH levels and GRC mediated by CREB1-NRF2 possessed improved self-renewal, migratory, anti-inflammatory, and T-cell suppression capacities. Administration of MSCs overexpressing CREB1-NRF2 target genes alleviated GVHD in a humanized mouse model, resulting in improved survival, decreased weight loss, and reduced histopathologic damages in GVHD target organs. Collectively, these findings demonstrate the molecular and functional significance of the CREB1-NRF2 pathway in maintaining MSC GSH dynamics, determining therapeutic outcomes for GVHD treatment.

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Keywords: Glutathione, Real-time monitoring, Graft-versus-host disease

Poster: 213

REFINING TOOLS FOR CRISPR/CAS9 TARGETING IN HPSC-CARDIOMYOCYTES

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Human pluripotent stem cell (hPSC)-cardiomyocytes (CMs) are now a bona fide component of many cardiac disease modelling, drug screening, and toxicology studies. Protocols have begun to emerge for both higher throughput 2D applications, as well as various complex 3D systems, however those exploiting CRISPR-based technologies are still nascent. Within the Technology Platform Pluripotent Stem Cells, we develop tools and workflows to facilitate basic and translational research using hPSCs and their derivatives. For hPSC-CMs, more established protocols for differentiation and cryopreservation are combined with new techniques for handling and manipulation. In particular, we are focussed on methods for CRISPR-based screening directly in hPSC-CMs. Using Cas9 and sgRNA, we show InDel formation at a range of different loci without deleterious effects on cell viability. We are testing various aspects including exogenous or AAVS1-transgene expression options for Cas9 to facilitate simple and robust targeting. These tools will ultimately enable higher throughput screening for novel targets such as those regulating proliferation, maturation, or cardioprotection against cytotoxic pharmaceutical compounds.

Funding Source: DZHK (German Centre for Cardiovascular Research)

Keywords: CRISPR/Cas9 screening, hPSC-CM, cardioprotection

Poster: 214

EMBRYONIC STEM CELL APPLICATIONS WITH NANOFIBRILLAR CELLULOSE AS A 3D CULTURE MATRIX

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Three-dimensional (3D) cell culture models are proving invaluable and necessary tools for drug testing, disease modelling, or studying tissue and organ development. These models are

needed to provide better biologic relevance, functionality to better mimic the in vivo conditions compared to traditional 2D in vitro cultures. Renal organoids are able to mimic the structure and function of in vivo kidneys. These organoids are needed for drug discovery testing or studying kidney development. More robust high throughput and scalable 3D models of kidney organoids are needed. Nanofibrillar cellulose (NFC) hydrogels, GrowDex® are animal free and tunable extracellular matrices derived from the birch tree, which support the culture of renal organoids. In this study, renal organoids were cultured from E11.5 primary mouse embryonic kidney metanephric mesenchymal (MM) cells followed by chemical induction to undergo nephrogenesis. They were either cultured for 5 days on top of a membrane without NFC in Trowell culture or embedded in GrowDex. NFC was used to provide a clean and well-defined matrix into which organoids could be embedded, reducing distortion or stress-induced affects during the nephrogenesis process. This allowed the organoids to grow in 3D, better mimicking more natural and physiological conditions. Immunocytochemistry and confocal imaging were used to assess organoid development and maturation. Kidney tubule formation was visualised with Pax2 whilst maturation visualised with Lotus Tetragonolobus Lectin (LTL). Pax2 and LTL staining showed multiple developing nephrons with mature proximal tubule formation similarly for organoids in both culture conditions. Due to the 3D conditions, the organoids grown in NFC were more spherical compared to the control 2D Trowell cultured organoids. Therefore, it can be seen that NFC is not toxic for the 3D culture of renal organoids, and nephrogenesis proceeds normally where organoids are more spherical when embedded in NFC than in the traditional Trowell cultures. These results suggest that GrowDex can be used for renal organoid culture where the wood-derived NFC provides a well-defined, tunable 3D culture environment for future model development of kidney organoids. Authors would like to thank Ulla Saarela and Seppo Vainio and for performing the experiments.

Keywords: Nanofibrillar Cellulose, Organoids, ESCs

Poster: 215

A NOVEL SERUM- AND CONDITIONED MEDIUM-FREE FORMULATION TO STANDARDIZE HUMAN INTESTINAL ORGANOID CULTURES BY IMPROVED REPRODUCIBILITY

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Current culture media for human intestinal organoids uses Wnt-3a protein provided in the conditioned medium (CM) of a commonly available cell line. The undefined nature of CM and batch variability hinder the usage of organoids for applications requiring greater reagent specification and reproducibility. To address this, we developed IntestiCult™-SF Organoid Growth Medium for the establishment and expansion of human intestinal organoids in conditioned medium- and serum-free conditions. Organoids were passaged every week and the average split ratio was 1:10 for small intestine (n=3) and 1:6.3 for colon (n=4) samples. IntestiCult™-SF supports the expansion of organoids generated from small intestinal and colonic biopsies from normal or polyp tissues for a minimum of 10 passages. Principal component analysis of bulk RNA-seq data shows similar gene expression profiles of both small intestinal and colon organoids grown in IntestiCult™-SF, control IntestiCult™ OGM (Human), and the original published formulation, indicating that all media formulations expanded similar cell populations. Intestinal epithelial cells organize into polarized cystic or complex structures resembling the crypt-villus morphology observed in conditioned medium-based formulations. Organoids maintained in IntestiCult™-SF expressed protein markers of intestinal stem cells (OLFM4), proliferative cells (Ki-67) and differentiated cells (FABP1, MUC2, and KRT20) by immunocytochemistry. The increased percentage of organoid formation (2-10%) from single, unsorted intestinal cells when cultured in IntestiCult™-SF compared to control (n=4), provides an efficient platform for CRISPR/CAS single-cell gene editing. Organoids were also subjected to differentiation protocols in 3D and 2D monolayers using IntestiCult™ Organoid Differentiation Medium (ODM). Organoids differentiated in 2D monolayers show a 10-fold decrease in expression of the stem cell markers LGR5 and ASCL2 and a 5-125-fold increase in expression of MUC2, APOB, and KRT20. Differentiation in 3D cultures leads to an increased expression of APOB, MUC2, CHGA (4-389-fold) while LGR5 and ASCL2 are maintained. IntestiCult SF is a new, single-component culture medium for the rapid expansion, bio-banking and subcloning of human intestinal organoids applications.

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Keywords: human-intestinal-organoids, IntestiCult, Serum-free

Poster: 217

SINGLE CELL SUSPENSION-BASED APPROACHES FOR UPSCALING INDUCED PLURIPOTENT STEM CELL CULTIVATION IN 3D BIOREACTORS

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Now in its second project phase, the European Bank for induced pluripotent Stem Cells (EBiSC) aims at long-term self-sustainability of the cell repository. With nearly 900 high quality iPSC lines in the catalogue, methods to support the cultivation of these cells at scale are currently undergoing development. Using an existing system of impeller-free suspension bioreactors maintained in a bench-top 3D cell culture incubator (CERO®), we sought to further intensify upscaling processes and increase protocol flexibility to suit individual needs. We assessed different approaches aimed at increasing cell yields. In contrast to seeding iPSCs as cell clumps, iPSCs were enzymatically dissociated into single cell suspensions for these approaches. In the first protocol, single cell suspensions were seeded with ROCK inhibitor (Y27632) in the absence of microcarriers, where

cells then proliferate as aggregates. In the second protocol, single cell suspensions were seeded with Matrigel-coated microcarriers and with ROCK inhibitor, where cells attach to the microcarriers and expand as layers on the microcarriers. After several passages of expansion with each single cell seeding protocol, cells were harvested and analysed for their quality. We demonstrate that the two single cell suspension-based protocols show robust cell expansion in the bioreactors. Cell quality was also assessed by key assays (e.g. flow cytometry and qRT-PCR for pluripotency-associated markers, post-thaw viability), suggesting that these protocols are viable alternatives to scale up the cultivation of iPSCs.

Funding Source: This project received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under agreement No 821362. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

Keywords: EBiSC, cell banking, bioprocessing

Poster: 218

INDUCED PLURIPOTENT STEM CELLS FROM THE FUNCTIONALLY EXTINCT NORTHERN WHITE RHINOCEROS

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The existence of roughly a third of all land vertebrate species is threatened by human activity. With only two females left alive, the northern white rhinoceros (NWR) is probably the earth's most endangered mammal. By combining stem cell biology with advanced reproduction technologies, we aim to rescue the functionally extinct species. Toward this end, we generate induced pluripotent stem cells (iPSCs) from cryopreserved tissue samples of already deceased NWR individuals, which subsequently will be differentiated into gametes, in vitro fertilized, and transferred into surrogates of the closely related, less threatened southern white rhinoceros (SWR). To ensure production of high quality iPSCs, we karyotyped source fibroblasts of five NWR individuals and one NWR-SWR hybrid, and determined their cell doubling time. We optimized integration-free reprogramming of NWR fibroblasts using human Sendai virus and generated iPSC lines from the deceased NWR individual Nabire using fibroblasts isolated from both ovary and skin. We characterized NWR iPSCs thoroughly and demonstrate their differentiation potential into beating cardiomyocytes (mesoderm), brain organoids (ectoderm), and endoderm. Currently, we are focusing on differentiating NWR iPSCs into primordial germ cells and generating iPSC lines from further NWR individuals using Sendai virus encoding rhinoceros reprogramming factors.

Funding Source: This work is supported by the German government (BMBF 01LC1902B BioRescue).

Keywords: stem cell associated technique (SCAT), species extinction, conservation

Poster: 220

LABEL-FREE FUNCTIONAL CHARACTERIZATION OF HUMAN BRAIN ORGANOIDS AT SINGLE-CELL RESOLUTION

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Three dimensional organ-like cell aggregates (organoids) that originate from human induced pluripotent stem cells (h-iPSCs) are emerging as promising tools for investigating development and disease progression, as well as for drug discovery. Organoids from h-iPSC-derived neurons recapitulate the architectures and characteristic functions of different brain areas that can be used to model human disease in-vitro. In order to adopt brain organoids for rapid and cost-effective drug screenings, it is necessary to assess their cell type composition, gene expression patterns and physiological function. The electrical activity of brain, retina or muscle organoids can now be easily captured, label free, at single-cell resolution by using high-density microelectrode array (HD-MEA) technology. The HD-MEA's large sensor array, featuring 26,400 electrodes at high-resolution enables recording of neuronal activity across different scales, from the network level, single-cell level, down to the sub-cellular level. Three different neuronal assays have been implemented and used to evaluate the spontaneous activity of brain organoids. (A) The ActivityScan Assay allows detection and identification of all active areas in the organoid. Firing rate and amplitude of the action potentials can be extracted. (B) The Network Assay enables the analysis of network bursts and synchronicity, indicating formation of connectivity between neurons. (C) The AxonTracking assay identifies single neurons and provides metrics such as axonal conduction velocity. In this work, we present results from organoids modeling different brain compartments and will demonstrate the potential of HD-MEA technology for characterizing the physiological function of human brain organoids and for testing compounds.

Keywords: brain organoids, high-density microelectrode array, electrophysiology

Poster: 221

UP TO 151 MILLION CUMULATED FOLD EXPANSION OF ENCAPSULATED HIPS CELLS IN BIOREACTOR OVER 28 DAYS, AND COMPARISON WITH 2D CULTURE AND STANDARD SPHEROID CULTURE.

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Stirred tank bioreactors constitute an obvious path to scale-up manufacturing of stem cell-based therapies to treat up to millions of patients with up to billions of cells each. Yet the mechanical agitation necessary to homogenize the media can negatively impact cell viability and phenotype. Here we propose to encapsulate human pluripotent stem cells (hPSCs) in

hollow alginate capsules to protect them from impeller-induced mechanical damage in bioreactors. Pluripotent stem cell encapsulation is performed at high-throughput - 1,000 capsules per second - using a proprietary microfluidic device designed to meet industrial requirements (GMP-ready, automated, closed and single-use system). Once encapsulated, cells grow in a protected microenvironment without direct contact with bioreactors mechanical stressors. Each micro-compartment allows for the self-organization of a biomimetic epiblast-like 3D stem cell colony. Following cell amplification inside the capsule, cells can easily be harvested by dissolving the hydrogel shell. Here we demonstrate: (A) A very robust weekly amplification factor: >100x in 7 days; (B) Similar performance in static or stirred cultures; (C) Straightforward scale-up from 3mL static culture to 1L bioreactor (10L pending); (D) Proven maintenance of stemness: >92% OCT4/NANOG coexpression in 4 iPS cell lines; (E) less than 2% total cell mortality over 7 days; (F) Serial encapsulation capacity: 4 encapsulations in a row, 151 000 000X cumulated amplification factor over 28 days of dynamic suspension culture in capsulo, >99 % OCT4/NANOG coexpression at day 28 in IMG005 line; In summary, high throughput production of scale-independent micro-environments ensures consistency of cellular parameters throughout the scale-up process. This integrated platform might de-risk and accelerate the clinical translation of cell therapies.

Funding Source: Research funded by TreeFrog Therapeutics. TreeFrog Therapeutics is receiving funding from the European Union's Horizon 2020 research and innovation program through the SME Instrument Phase 2 under grant agreement n° SME 881113.

Keywords: iPS, Bioreactor, Pluripotent stem cell

Poster: 222

MICROFLUIDIC HIGH-THROUGHPUT SCREENING PLATFORM TO SCREEN PRE-CLINICAL STAGE COMPOUND EFFECTS ON NEURITE OUTGROWTH OF HUMAN MOTOR NEURONS POST INJURY

Rontard, Jessica, BATUT, Aurélie, Gleyzes, Mélanie, Debis, Delphine, Margot, Libralato, Dubuisson, Louise, Vieira, Janaina, Calderini, Yannick, Larramendy, Florian, Honegger, Thibault

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Traumatic spinal cord injury (SCI) affects millions of people of all ages around the world and can potentially lead to irreversible cognitive and motor damage without any immediate therapeutics available. There is a significant demand for physiologically relevant models of SCI. The rise of Organ-on-Chip technologies combined with human cells opens new route to limit the number of animal experiments. The major modeling challenge is to accurately perform the injury only on the neurites without affecting cell viability and to apply the pre-clinical stage compounds on cell soma only. We will present the design and fabrication of a compartmentalized microfluidic device with three compartments capable of both inducing a localized axotomy and a fluidly isolated cell chamber. The device allows quantification of axonal regeneration post-injury using an innovant and unique triangular design. Human-induced pluripotent stem cell-derived motor neurons are seeded and maintained up to 3 weeks in vitro. We performed a chemically-induced axonal injury on isolated axons in the middle compartment only. We then follow the dynamics of the axonal regeneration using the triangular methodology with and without tested neuropharmacological compounds in soma chamber. We show that this model can be successfully used for quantitative analysis of neurite outgrowth dynamics. We will finally present how this model can be used to record the functional activity using multielectrode arrays (MEA) coupled to the microfluidic device. The access to functional data during the injury and the recovery process provides relevant insight on the mode of action of pharmacological compounds. Our data suggest that this model can be used for high-throughput drug-induced axonal regeneration screening for preclinical stages pharmaceutical compounds.

Keywords: Axonal regeneration, Microfluidics, Spinal Cord Injury

00:00 - 1:00 EDT

POSTER SESSION 2

TISSUE STEM CELLS AND REGENERATION

Poster: 232

CDH18 IS A NOVEL BIOMARKER FOR HUMAN FETAL EPICARDIUM REGULATING DIFFERENTIATION TOWARDS CARDIAC VASCULAR SMOOTH MUSCLE CELLS

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The epicardium is a mesothelial layer covering the myocardium and serves as a progenitor source supporting cardiac development, repair and regeneration. During development the active epicardium contributes to different cardiac lineage descendants, being essential for cardiogenesis. Interestingly, the epicardium also reactivates after cardiac injury, playing a major role during tissue remodeling and cardiac regeneration. Fine-tuned balanced signaling regulates cell plasticity and cell-fate decisions of epicardial-derived cells (EPCDs) via epicardial-to-mesenchymal transition (EMT). In recent years the epicardium has emerged as a therapeutic target, however epicardial biological as well as repair and regeneration processes are still poorly understood as powerful tools to investigate epicardial function, including markers with pivotal roles in developmental signaling, are lacking. Here, we recapitulated epicardiogenesis using human induced pluripotent stem cells (hiPSCs) and identified type II classical cadherin CDH18 as a novel biomarker defining lineage specification in human active epicardium. The loss of CDH18 led to loss of epicardial identity accompanied by the onset of EMT. Furthermore, activation of Wnt signaling pathway in combination with loss of TCF21 upon CDH18 silencing resulted in a cell-fate and differentiation towards cardiac vascular smooth muscle cells. We found this effect to be more apparent in cells representing a more pro-epicardial state compared to cell representing a fetal-like epicardial stage. We also showed that, GATA4 is a putative regulator of epicardial CDH18 expression. These results highlight the biological implications of tracing CDH18 expression in hiPSC-derived epicardial cells for a wide range of application ranging from cell enrichment to engineering of cell-fate, thus providing a model

for investigating epicardial function in human development and disease and enabling new possibilities for regenerative medicine.

Keywords: epicardium, EMT, CDH18

Poster: 234

A CALCINEURIN-MEDIATED SCALING MECHANISM THAT CONTROLS A K+-LEAK CHANNEL TO REGULATE MORPHOGEN AND GROWTH FACTOR TRANSCRIPTION

Antos, Christopher L.[1], Chao, Yi[1], Spitters, Tim[1], Al-Far, Ezz[2], Wang, Sen[1], Cai, Simian[1], Yan, Xin[1], Guan, Kaomei[2], Wagner, Michael[5], El-Armouche, Ali[2]

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All animals grow organs and appendages to the exact same dimensions. The control of proportional growth is a fundamental yet poorly understood phenomenon that involves the coordinated scaling of all the stem and progenitor cells of each tissue within the organ. Previous findings show that the continued activity of the two-pore potassium-leak channel Kcnk5b maintains allometric juvenile growth of adult zebrafish appendages. However, it remains unknown how this channel maintains allometric growth and how its bioelectric activity is regulated to scale these anatomical structures. We show the activation of Kcnk5b is sufficient to activate several development programs in adult, larva and embryonic structures. The most responsive of which are Shh and Lef1. We provide in vivo transplantation evidence that the activation of these developmental programs is cell autonomous. We also show that this bioelectric signal can induce the expression of different subsets of developmental genes using different cultured mammalian cell lines, indicating that the electrophysiological changes induced by Kcnk5b are not restricted to specific developmental cascades. We also provide evidence that the post-translational modification of serine 345 in Kcnk5b by calcineurin regulates channel activity and controls the fin developmental programs to scale the entire fin anatomical structure. Thus, we show how an endogenous bioelectric program can be regulated to coordinated different developmental signals to generate and scale a vertebrate appendage.

Funding Source: Deutsche Forschungsgemeinschaft Grant AN 797/4-1 Shanghaitech University

Keywords: proportional growth, electrophysiology, zebrafish appendages

Poster: 235

INDUCTION OF HEMATOPOIETIC STEM CELLS FROM MOUSE EMBRYONIC PRE-HSCS IN SERUM-FREE AND FEEDER-FREE CULTURE SUPPLEMENTED WITH SCF AND TPO

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Hematopoietic stem cells (HSCs) are produced from hemogenic endothelial cells (HECs) through an intermediate stage known as the precursor of HSC type I (pre-HSC-I). Several studies have reported that pre-HSC-I differentiate into HSCs when cultured in various conditions such as co-culture with stromal cells. However, the presence of feeder cells and fetal calf serum has hampered the understanding of essential molecules that regulate HSC development. In this study, we aimed at elucidating minimal growth factors required for supporting the HSC differentiation from pre-HSC-I. Pre-HSC-I isolated from E11.5 mouse embryos were cultured in a serum-free and feeder-free condition supplemented with stem cell factor (SCF) and thrombopoietin (TPO). SCF and TPO synergistically accelerated cell proliferation and increased c-Kit+Sca-I+lineage- (KSL) cells. Only the combination of SCF and TPO induced HSCs with an engraftment ability. These data indicate that both SCF and TPO are required for cell proliferation and HSC development from pre-HSC-I in culture. Single-cell RNA-seq analyses of the AGM region and the fetal liver of E10.5 and E11.5 embryos showed that pre-HSC-I expressed SCF receptor, c-Kit, but not TPO receptor, Mpl. In contrast, pre-HSC-II, the descendant of pre-HSC-I, expressed both receptors, suggesting that TPO is effective only after the pre-HSC-II stage. Previous studies have noted that the deletion of the Tpo gene has no impact on HSC development in the fetal liver. Some compensatory cytokines might support HSC generation in the embryo, while TPO is suggested to be required for the in vitro differentiation of HSCs from pre-HSCs. Our findings will open up new possibilities for the derivation of transplantable HSCs from pluripotent stem cell lines.

Keywords: hematopoietic stem cells, in vitro culture, pre-HSCs

Poster: 236

BMP4 PROMOTES THE DISTINCT VE-CADHERIN EXPRESSING PROGENITORS IN THE DEFINITIVE HEMATOPOIETIC DIFFERENTIATION FROM MOUSE EMBRYONIC STEM CELL-DERIVED LATERAL MESODERM

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Definitive hematopoietic precursors, including hematopoietic stem cells (HSCs), develop from the fetal liver kinase 1 (Flk1)-expressing lateral mesoderm by passing through several types of intermediate progenitor cells, e.g., VE-cadherin+ CD41- CD45- (V+41-45-) hemogenic endothelial cells (HECs) and VE-cadherin+ CD41+ CD45- (V+41+45-) cells. Identifying the signaling molecules that regulate the development of definitive hematopoietic precursors from the lateral mesoderm is key to achieving the generation of HSCs from pluripotent stem cells. Bone morphogenetic protein 4 (BMP4) is essential for mesoderm formation. However, the function of BMP4 in the differentiation of definitive hematopoietic progenitors from the lateral plate mesoderm has been unclear. We examined the impact of BMP4 on the definitive hematopoietic development from the lateral mesoderm by using an in vitro differentiation system of mouse embryonic stem cells (ESCs). Co-aggregation culture of ESC-derived Flk1+ mesodermal cells with OP9 stromal cells induced V+41-45- HECs and V+41+45- cells. The induced cells had the potential of erythro-myeloid and T lymphoid differentiation. The addition of a high concentration of BMP4 increased V+41-45- HECs, thereby leading to the subsequent increase of progenies,

i.e., V+41+45- hematopoietic progenitor cells and CD45+ hematopoietic cells. Furthermore, the expansion of V+41+45- hematopoietic progenitor cells was also enhanced by BMP4. The addition of BMP4 increased the hematopoietic colony-forming cells of multiple lineages. These results suggest that BMP4 has promotive effects on the differentiation of HECs and V+41+45- hematopoietic precursors from lateral mesodermal cells. These findings should help the establishment of in vitro culture system to induce HSCs from ESCs.

Funding Source: This work was supported by JSPS KAKENHI Grant Numbers 15K07081 and 18K06262 (MO).

Keywords: Bone morphogenetic protein 4, Hemogenic endothelial cells, Embryonic stem cells

Poster: 237

HEAT TREATMENT FACILITATES DIFFERENTIATION OF HEPATOCYTE-LIKE CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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Human pluripotent stem cell-derived hepatocyte-like cells (hPSC-HLCs) are considered as promising cells for resolving the problems faced in using primary hepatocytes in liver research so far, such as difficulties in growth and maintenance, and limited number of donors. Previous differentiation methods involve the introduction of growth factors and scaffolds, but hPSC-HLCs are still immature, and therefore, further methods are required to be developed. To resolve this issue, we focused on the temperature at which hepatocytes naturally exist under physiological conditions. Liver is a heat-producing organ, and it is known that the physiological temperature of liver is higher than the general cell culture temperature (37°C). Therefore, it is likely possible that heat stimulation is involved in the differentiation of hepatocytes. In this study, we examined the effect of heat treatment on hPSC-HLCs undergoing differentiation. First, to determine the temperature suitable for heat treatment, we examined cell viability, and found that hPSC-HLCs were viable at 39°C, whereas many cells died at 42°C. Therefore, the temperature for heat treatment was set to 39°C. Second, to confirm the effect of heat treatment on hPSC-HLCs, we examined the expression of hepatic markers after 12 days of treatment. Cells treated at 39°C exhibited an increase in albumin secretion and the number of albumin-positive cells, and α1-antitrypsin-positive cells, as well as increased activity of cytochrome P450 3A (CYP3A), a drug-metabolizing enzyme, and CYP3A- positive cells. Finally, to elucidate the mechanism underlying the increase in hepatic differentiation, we conducted RNAseq analysis. In cells treated at 39°C, the expression of genes related to protein folding and the organization of extracellular matrix that supports hepatocytes was found to be increased, suggesting that the activation of these genes might be involved in facilitating the differentiation of hepatocytes. These results indicate that heat treatment can be utilized as an important tool for promoting hepatic differentiation. Since using our method, we could improve the differentiation of hepatocytes simply through changing the culture temperature, it is possible to produce mature hepatocytes through combining it with other differentiation methods used so far.

Funding Source: The Japan Society for the Promotion of Science (16K14660, 17H02083, 18KK0306 and 19H02572), The Japan Agency for Medical Research and Development (17937667) The LiaoNing Revitalization Talents Program (XLYC1902061).

Keywords: Heat treatment, Hepatocyte, differentiation

Poster: 238

FGF AND WNT SIGNALING INTERACTION IN THE MESENCHYMAL NICHE REGULATES THE MURINE HAIR CYCLE CLOCK

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Tissue growth in the adult is an orchestrated process that often requires biological clocks to time stem cell and progenitor activity. Here, we employed the hair follicle, which cycles between growth and regression in a timely-restricted mode, to show that some components of the hair cycle clock reside within the mesenchymal niche of the hair follicle, the dermal papilla (DP), and both Fgf and Wnt signaling pathways interact within the DP to regulate the expression of these components that include Wnt agonists (Rspondins) and antagonists (Dkk2 and Notum). The levels of Wnt agonists and antagonists in the DP are progressively reduced and elevated during the growth phase, respectively. Consequently, Wnt signaling activity in the overlying epithelial progenitor cells decreases, resulting in the induction of the regression phase. Remarkably, DP properties allow Wnt activity in the DP to persist despite the Wnt-inhibiting milieu and consequently synchronize the induction and progression of the regression phase. This study provides insight into the importance of signaling crosstalk in coupling progenitors and their niche to regulate tissue growth.

Keywords: Hair follicle, Wnt signaling, Skin

Poster: 239

HUMAN FETAL TISSUE RESEARCH AND ABORTION DISPUTE IN JAPAN

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The Trump administration introduced an extremely suppressive policy, supported by pro-life activists, on human fetal tissue (HFT) research. In the US, the dispute on HFT research has been almost parallel with the abortion dispute. In this background, this presentation considers the relationship between HFT research and abortion disputes in Japan. The Japanese government legalized abortion in 1948. Since then, there have been no severe abortion disputes in the country. However, in the 1970s and 1980s, the policy to restrict elective abortion (the wording of the law is “abortion for economic reasons”) was discussed in the Diet led by Diet members who were supported by the religious group, Seicho-No-Ie (The House of Infinite Life, Wisdom and Abundance) claiming respect for life. In the end, the “economic reasons” remained, partly because of the feminist activities that supported elective abortion as women’s choices. This was the only notable abortion dispute in Japan, and much milder than that observed in the US. Although there has been no clear rule about HFT research, few researchers in Japan have

undertaken HFT studies recently, due to "ethical reasons". In the 2000s, a government committee discussing a guideline for clinical research using human stem cells contemplated on the conditions for the use of the HFT, but the issue of the HFT itself was excluded from the guideline due to various factors. One of the committee members belonged to a feminist group that supported elective abortion. This feminist group has been active since the 1980s. Considering the negative impacts that the HFT donation procedures have on women undergoing abortion procedure, she and her group opposed HFT research. However, those who were against elective abortion from the 1970s rarely participated in the controversy on HFT research. Thus, unlike in the US, the dispute on HFT research was not parallel with Japan's abortion dispute.

Funding Source: This research was supported by AMED under Grant Number JP21bm0904002.

Keywords: Human Fetal Tissue, Elective Abortion, Abortion Dispute

Poster: 240

LOCALIZATION OF ADULT TISSUE-RESIDENT STEM CELLS IN NATIVE HUMAN RETINAL PIGMENT EPITHELIUM

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Retinal Pigment Epithelium (RPE) is a monolayer of post-mitotic, pigmented cells beneath neural retina, which is actively involved in the visual process by supporting the replenishment of photoreceptor outer segment. Dysfunction of RPE is associated with age related macular degeneration, a leading cause of blindness worldwide. In human RPE, stem cells (RPE-SCs) have been identified only in cultured cells. But no report is available on the stem cells in native RPE, their role in maintaining tissue homeostasis and in the pathology of age related macular degeneration. The aim of this study is to identify the location of stem cells in native human RPE. RPE was demarcated into three equal concentric regions centered on the optic nerve head: central, equatorial and peripheral region. Whole mount of human RPE and RPE sections were immunostained for the proliferation marker Ki67 and stem cell markers (KLF4, OCT4, SOX2 and NANOG). RPE cells in the peripheral region were observed to express Ki67 and such proliferating cells were absent in the central and equatorial regions. The expression of stem cell markers was negative in all regions. Real time PCR analysis for the same panel of stem cell markers with cells isolated from different regions of RPE identified a 2.14-fold higher expression of embryonic stem cell marker OCT4 in peripheral RPE compared to central and equatorial RPE while the expression of other markers were similar. Further, analysis of the native RPE cells for neurosphere formation, a functional property of adult stem cells indicated that cells from the peripheral region alone had the ability to generate neurospheres. The above results indicate that the adult human RPE-SCs might be located in the peripheral RPE which requires further confirmation.

Funding Source: Council of Scientific and Industrial Research (CSIR), India, provided Junior Research Fellowship

Keywords: RPE, adult tissue resident stem cells, proliferation

Poster: 241

SEARCHING FOR FACULTATIVE CELLS THAT ASSIST WITH LONG-BONE CATCH-UP GROWTH IN MICE

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Developmental robustness is the ability to maintain organ size, shape and function, despite the presence of perturbation. Loss of this capacity may lead to altered body proportions after a local injury, affecting quality of life. The human body can recover from injuries in early development but this capacity declines postnatally, a decline that is not fully understood. We study this topic by analyzing how limbs recover a normal growth trajectory after a developmental insult in mouse, which is known as catch-up growth (CUG). We can induce the injury unilaterally (left limb) and in a transient manner. We successfully generated transient injury in the cartilage regions that form the scaffolds for future long bones, creating left-right limb asymmetry 3 days post-injury. This was done by transient expression of Diphtheria Toxin A (DTA) exclusively in the left limb chondrocytes, leaving the right limb as internal control. Our results showed that 6 days and 13 days post-injury, the left limb showed signs of recovery where the length of the left limbs was catching up with the contralateral ones. Based on lineage-tracing studies, we hypothesized that there is a transient backup cell population adjacent to the cartilage that does not normally contribute much to bone growth, but that gets activated in response to injury and contributes to the repair. Indeed, we found that components of the immediate-early response pathways (Egr1, c-Fos) are activated shortly after injury, suggesting an 'alarm call' is triggered. Since several studies have shown that mTORC1 pathway is critical for stem cell activation in multiple organs, we characterized the spatiotemporal expression of phosphorylated ribosomal protein S6, a readout of mTORC1 activity. We found that it is upregulated in a wave that seems to invade the left cartilage from adjacent tissues. Future experiments aim to identify the exact cells and molecules involved, to provide the groundwork for new treatments for growth disorders.

Funding Source: NHMRC APP2002084 & HFSP CDA-00021/2019-C

Keywords: Adult stem cell, Cartilage regeneration, Bone development

Poster: 242

NRP2+ HUMAN MESENCHYMAL STEM CELLS HAVE STEMNESS-ASSOCIATED PROPERTIES

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Mesenchymal stem cells (MSCs), which can self-renew and differentiate into bone, cartilage, and fat, have attracted attention for their clinical applications. Although it is essential to isolate undifferentiated, non-senescent MSCs for the clinical application of MSCs to human regenerative medicine, specific markers for undifferentiated, non-senescent MSCs have not yet been identified. We previously established a method to isolate and clone MSCs from human bone marrow by flow cytometry using two antibodies against low-affinity nerve growth factor receptor and Thy-1 and classified fast-growing clones as Rapidly Expanding Clones (RECs) and slow-growing clones as Moderately Expanding clones (MECs). RECs exhibited robust multilineage differentiation and self-renewal potency compared with MECs. We therefore consider RECs to be the most primitive and undifferentiated population. In the present study, to identify the genes specifically expressed in undifferentiated, non-senescent MSCs, a comprehensive gene expression analysis of RECs and MECs was performed and revealed that Neuropilin-2 (NRP2) was strongly expressed in RECs. We then found that RECs included NRP2+ and NRP2- clones. NRP2+ clones exhibited a stronger potential for proliferation and differentiation into osteocytes and adipocytes than NRP2- clones. NRP2+ clones also had a stronger migratory capacity than NRP2- clones. Furthermore, VEGF-C/NRP2 signaling enhanced proliferation and differentiation abilities. These results suggest that NRP2 has potential as a cell surface marker for MSCs with stemness properties. Therefore, we expect that NRP2+ MSCs will be applied to human regenerative medicine in the future.

Keywords: Human mesenchymal stem cell, Neuropilin-2, Regenerative medicine

Poster: 243

ATTENUATION OF TRAUMATIC-INDUCED OSTEOARTHRITIS BY REPETITIVE INTRA-ARTICULAR ADMINISTRATION OF PERIPHERAL BLOOD-DERIVED MESENCHYMAL STEM CELLS

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Osteoarthritis (OA) is a chronic joint disease, characterized by articular cartilage degradation, subchondral bone hardening, and inflammation of the whole synovial joint. There is no pharmacological treatment in slowing down OA progression, leading to costly surgical interventions eventually. Cell therapy using various cartilage and progenitor cell types from different sources has been reported in clinical trials for OA management with some success, but outcomes are varied. Peripheral blood derived circulating mesenchymal stem cells (PB-MSCs) are promising cells owing to their easy collection, superior migration and differentiation potentials. In the current study, we evaluated the effect of intra-articular administration of PB-MSCs on the progression of OA in mice. PB-MSCs and AD-MSCs were harvested and cultured according to previous published protocols, and pre-labeled with BrdU for 48 h before use. PB-MSCs/AD-MSCs (5×105 cells/mouse; passage 3~5) were injected into the right knee joints thrice every-other-week post-surgery (except sham surgery group). The mice were terminated at 8 weeks post-surgery and knee joint samples were collected for micro-CT and histology examinations. PB-MSCs administration significantly reduced hardening of subchondral bone comparing to vehicle controls. Safranin O staining showed that PB-MSCs treatment ameliorated degeneration of articular cartilage, which is comparable to AD-MSCs treatment. The expression of MMP13, collagen X, and IL-1β was significantly reduced on articular cartilage of PB-MSCs-treated groups comparing to vehicle controls. Co-immunofluorescence analysis for BrdU and Sox9 detection further confirmed differentiation of PB-MSCs injected towards chondrocytes. The ELISA assay showed reduced concentrations of IL-6 and IL-1β in the peripheral sera of PB-MSCs- and AD-MSCs-treated mice. Therefore, repetitive administration of PB-MSCs halted OA progression through inhibition of cartilage degradation and stimulation of cartilage regeneration. PB-MSCs may become a promising cell source for cartilage tissue repair and OA prevention.

Funding Source: Hong Kong Government Research Grants Council, Collaborative Research Fund (C7030-18G), General Research Fund (19-093-GRF, 14120118, 9054014, N_CityU102/15, and 14119115), Hong Kong ITF (PRP/050/19FX and ITS/448/18).

Keywords: osteoarthritis, mesenchymal stem cells, inflammation

Poster: 244

JMJD1A REGULATES ADULT HIPPOCAMPAL NEUROGENESIS AND BRAIN INJURY REPAIR

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Newborn hippocampal neurons, generated by the neural stem/progenitor cells (NSPCs), are important for cognitive functions and mood control. During aging, deterioration of NSPCs leads to compromised neurogenesis and age-related cognitive decline and psychiatric disorders. Jmjd1a, a histone demethylase, demethylates mono and dimethyl lysine 9 of histone H3. Activation of Jmjd1a leads to a loss of H3K9 repressive mark, allowing transcriptional activation of the Jmjd1a target genes that are involved in either physiological or pathological conditions such as spermatogenesis, mammalian sex determination, lipid metabolism and cancer development. Despite these findings, the function of Jmjd1a in the brain and particularly neurogenesis is largely unknown. We found that Jmjd1a was mainly expressed in the hippocampus, especially the dentate gyrus, CA1 and CA3. In the postnatal mouse hippocampus, Jmjd1a was largely expressed in the neuroblasts and immature neurons, indicating Jmjd1a is closely associated with hippocampal neurogenesis. Loss of Jmjd1a in mice led to a marked reduction in neuroblasts, immature neurons and mature neurons at postnatal stage. Consistently, KO NSPCs showed a compromised proliferation and neuronal differentiation potential in vitro. Mechanistically, we observed that loss of Jmjd1a resulted in a reduction of active-β-catenin and β-catenin expression in the subgranular zone of dentate gyrus. Further characterization using NSPCs unveiled that Jmjd1a destabilized CK1α, loss of which led to the accumulation of CK1α which subsequently aggravated the degradation of b-catenin and downregulation of b-catenin downstream targets. The regulatory role of Jmjd1a in neurogenesis was validated in the Nestin-cre; Jmjd1a-KO mice. Moreover, both whole body KO mice and Nestin-cre; Jmjd1a-KO mice showed deficit in spatial learning and memory at adult stage. Finally, in a controlled cortical injury model, injury-induced neurogenesis was significantly alleviated in Nestin-cre; Jmjd1a-KO mice. Collectively,

we reveal that Jmjd1a regulates normal function of NSPCs and adult neurogenesis of the brain via its interaction with CK1± which could regulate the activation of Wnt /^{2}-catenin pathway.

Funding Source: This research is supported by GRF14165217 and NSFC31970815

Keywords: Jmjd1a, neural stem cells, neurogenesis

Poster: 245

NEURONAL DIFFERENTIATION OF HUMAN STEM CELLS FROM APICAL PAPILLA USING RAT BRAINSTEM SLICES CONDITIONED MEDIUM TREATMENT

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According to the mammalian cochlear lacks the capacity to regenerate the cochlear hair cells and spiral ganglion neurons, the degeneration of these cells is regrading to be the sensorineural hearing loss. Additionally, there are no biological therapies to rescue the dying sensorineural cells or regenerate these cells to improve hearing ability. Previous studies also exhibited the possibility of various types of stem cells to differentiate into auditory neurons. Recent studies reported the potential of the stem cells-derived dental origin by using exogenous neurotrophins for neuronal differentiation into the spiral ganglion-like neurons. In the present study aim to demonstrate neuronal differentiation of the human stem cells from apical papilla (hSCAPs) by using conditioned medium collected from rat auditory brainstem slices (ABS-CM) that contains neurotrophic factors involved in their survival and regeneration of neurons. The experiments were performed the ABS-CM treatment for 2 and 3 weeks with 1:1 ratio of ABS-CM/ αMEM 1% Penicillin/Streptomycin compared to the negative control medium (αMEM 1% Penicillin/Streptomycin). After 2 and 3 weeks of culture, the neuronal differentiation of hSCAPs were analyzed on morphological observation, gene expression levels of NES, NF-M, MAP-2, and TUBB3, as obtained through the qRT-PCR, and immunofluorescence imaging. The results indicated that ABS_CM has the capability to induce neuronal differentiation of the hSCAPs after a minimum of 2 weeks of culture. Essentially, our preliminary findings demonstrated the possibility of using hSCAPs as an autologous stem cell-based therapy for patients suffering from sensorineural hearing loss.

Funding Source: This work was partially supported by Central Instrument Facilities (CIF), Faculty of Science, Mahidol University, Thailand.

Keywords: Neuronal differentiation, Human stem cells from apical papilla, Rat auditory brainslices conditioned medium

Poster: 246

SOX2 IS REQUIRED INDEPENDENTLY IN BOTH STEM AND DIFFERENTIATED CELLS FOR MOUSE PITUITARY TUMORIGENESIS IN P27-NULL MICE

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P27, a cell cycle inhibitor, is also able to drive repression of Sox2. This interaction plays a crucial role during development of p27−/− pituitary tumors because loss of one copy of Sox2 impairs tumorigenesis. However, SOX2 is expressed in both endocrine and stem cells (SCs), and its contribution to tumorigenesis in either cell type is unknown. We have thus explored the cellular origin and mechanisms underlying endocrine tumorigenesis in p27−/− pituitaries. We found that pituitary hyperplasia is associated with reduced cellular differentiation, in parallel with increased levels of SOX2 in stem and endocrine cells. Using conditional loss-of-function and lineage tracing approaches, we show that SOX2 is required cell autonomously in p27−/− endocrine cells for these to give rise to tumors, and in SCs for promotion of tumorigenesis. This is supported by studies deleting the Sox2 regulatory region 2 (Srr2), the target of P27 repressive action. Single cell transcriptomic analysis further reveals that activation of a SOX2-dependent MAPK pathway in SCs is important for tumorigenesis. Altogether, our data highlight different aspects of the role of SOX2 following loss of p27, according to cellular context, and uncover an unexpected SOX2-dependent tumor-promoting role for SCs. Our results imply that targeting SCs, in addition to tumor cells, may represent an efficient antitumoral strategy in certain contexts.

Keywords: pituitary, stem cell, tumorigenesis

Poster: 247

CHAPERONE-MEDIATED AUTOPHAGY MAINTAINS HUMAN GLIOBLASTOMA STEM CELL ACTIVITY THROUGH DIFFERENT PROTEOMIC AND TRANSCRIPTOMIC PATHWAYS

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Chaperone-mediated autophagy (CMA) is a homeostatic process essential for the lysosomal degradation of individual proteins. CMA activity directly depends on the levels of LAMP2A, critical receptor at the lysosomal membrane, to which CMA substrate proteins are bound. In glioblastoma (GBM), the most common and aggressive brain cancer in the adulthood, high levels of LAMP2A have been linked to TMZ resistance and tumor-associated pericytes. However, the implication of LAMP2A, and hence CMA, in glioblastoma stem cells (GSCs) remains unknown. In this work, we show that LAMP2A expression is enriched in GSC population and its downregulation diminishes GSCs tumorigenic activities. Proteomic and transcriptomic analysis of LAMP2A

downregulated patient-derived GSCs revealed extracellular matrix interaction effectors downregulated in both analyses. Moreover, interferon, antigen presentation and mitochondrial pathways were remarkably deregulated at proteome level, whereas PI3K-AKT and p53 pathways were altered in RNAseq study. Interestingly, these molecular changes were accompanied by a reduction in cytokine secretion, mitochondrial functionality and invasion and migration capacities. Clinical samples of GBM tissue presented upregulated LAMP2A expression, correlation with markers of pathways altered in proteome and transcriptome studies and its high levels correlate with advanced glioma grade and poor overall survival. In conclusion, we identified a novel role of CMA directly regulating GSCs activity via multiple pathways at proteome and transcriptome level.

Keywords: 'Glioma Stem Cells', 'Chaperone-mediated autophagy', 'High-throughput omic approach'

Poster: 248

PROMOTING THE DIFFERENTIAL PROCESS OF IADMSCS INTO NEURONAL CELLS THROUGH PHOTOBIOMODULATION

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The mammalian nervous system is very restricted in its repairing abilities in the event of mechanical injury or the onset of neurodegenerative disease, thus assistance is needed to optimize this regenerative process. One such strategy exists in the form of adipose-derived mesenchymal stem cells (ADMSCs) which can transdifferentiate into various cell lineages, including the neuronal kind. It has been suggested that this process can be optimized through the application of photobiomodulation (PBM). The aim of this in vitro study was to transdifferentiate ADMSCs with growth factors and chemical inducers and to subsequently determine the supporting effects of single use PBM at visible and near-infrared (NIR) wavelengths at a low fluency on the transdifferentiation process. iADMSCs were characterized through immunofluorescence, flow cytometry and ELISA to identify specific neuronal markers and transcription factors. Biochemical analysis involved observing morphology, migration rates, viability, proliferative effects, cytotoxicity, mitochondrial membrane potential, and generation of reactive oxygen species. Possible outcomes will be the successful differentiation of ADMSCs into neuronal cells through inductive means with the addition of PBM. Furthermore, an optimized protocol will be established for in vivo and clinical research specifically targeting neuronal regeneration. Findings from this research will serve as contribution toward validating stem cell technology for application in in vivo, pre-clinical and clinical research settings.

Funding Source: This research is supported by the GES 4.0 Postgraduate Scholarship received by MJVR; the NRF S&F -Scarce Skills Postdoctoral Fellowship (Grant no: 120752) received by AC; and the SARChI/NRF-DST (Grant no: 98337) received by HA.

Keywords: Adipose-derived mesenchymal stem cells, Photobiomodulation, Transdifferentiation

Poster: 249

EXPRESSION SIGNATURE AND REGULATORY ROLE OF MIRNAS IN HEPATOCELLULAR CARCINOMA-INDUCED CANCER ASSOCIATED FIBROBLASTS

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Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver worldwide. Mesenchymal stromal cells (MSCs) have shown promise in treatment of liver diseases and supporting liver regeneration. However, when MSCs are recruited from bone marrow to hepatic site of injury, abnormal expression of certain genes may be associated with a cancerous phenotype. Our study aims to assess the influence of soluble factors from HCC on MSCs and to investigate the regulatory effect of miRNAs in human adipose- MSCs (hA-MSCs) cultured in HCC microenvironment. Co-cultured hA-MSCs showed downregulation of MSC markers (CD 90 and CD 105) and the cancer marker K-RAS. Cancer associated fibroblasts (CAF) markers, α-SMA, Vimentin, c-MYc, MMP2, VEGF, IL-6, FGFR1, IL-8, SDF-1, and Tenascin-C were significantly upregulated. Significant upregulation in pluripotency markers NANOG and OCT4 was also observed. However, there was a significant downregulation in mRNA differentiation markers including ectodermal GFAP, mesodermal MSX1 and endodermal SOX-17 markers. No significant change in epithelial to mesenchymal transition markers. Significant upregulation in proliferation and cell cycle control gene CDK6, but not in CDK4 and E2F3 concomitant with upregulation in epithelial cell adhesion molecule EPCAM and CD44, but not in CD133. Co-cultured (CAF-like) cells showed decrease in cells percentage in S phase and appearance of aneuploidy peak. Functional enrichment analysis of deregulated proteins in CAF-like cells including our selected miRNA targets showed dysregulation of pathways and biological processes significantly involved in development of CAF characteristics. These include Wnt signaling and metabolic pathways and other processes contributing to CAF roles in cancer progression. Results suggest an interaction between tumor cells and surrounding stromal components and provide evidence to generation of CAF phenotype upon exposure of hA-MSCs to Huh-7 cancer microenvironment, favoring cancer progression. This might shed the light on fate of hA-MSCs use in HCC therapy. hA-MSCs modulation may be achieved via dysregulation of miR17-5P and 615-5p expression, suggesting that miRNAs may present a new target for HCC treatment.

Funding Source: This work is supported by grant #5300, funded by the Science and Technology Development Fund (STDF), Egypt to NE-B. and Zewail City of Science and Technology internal fund (2019-003).

Keywords: Adipose mesenchymal stromal cells, Cancer associated fibroblasts-like cells, Hepatocellular carcinoma

Poster: 250

COMPARATIVE PROTEOMIC ANALYSIS OF NUCLEAR AND CYTOPLASMIC COMPARTMENTS IN HUMAN CARDIAC PROGENITOR CELLS. FUNCTIONAL EVALUATION OF IL1A AND IMP3

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Clinical trials evaluating cardiac progenitor cells (CPC) demonstrated feasibility and safety, but no clear functional benefits. Therefore, a deeper understanding of CPC biology is warranted to inform strategies capable to enhance their therapeutic potential. Here we have defined, using a comparative label-free proteomic approach, the differential cytoplasmic and nuclear compartments of human CPC (hCPC), previously evaluated in an allogenic phase I/IIa, randomized and double-blind clinical trial (CARE-MI; EudraCT 2013-001358-81), for the treatment of patients with large cardiac infarcts. Global analysis of hCPC cytoplasmic repertoire suggested an important hypoxia response capacity and active collagen metabolism. In addition, analysis of the nuclear protein compartment identified a significant regulation of a small number of proteins in hCPC versus human mesenchymal stem cells (hMSC), used as reference. Two proteins significantly upregulated in the hCPC nuclear compartment, IL1A and IMP3 (IGF2BP3), were studied further. We found that IL1A, subjected to an important post-transcriptional regulation, upon induction of apoptosis, coupled to oxidative stress, was clearly upregulated and a substantial nuclear fraction was found. Therefore, it was demonstrated that IL1A act as a dual-function cytokine with a plausible role in apoptosis regulation. On the other hand, in homeostasis, we showed a clear fraction of IMP3 in the nuclear compartment of hCPC and apoptosis induction also provoked an enrichment of the nuclear fraction. Moreover, although the knockdown of the mRNA binding protein (IMP3) did not negatively impact hCPC viability, it reduced their proliferation and migration capacity. Finally, an analysis of a panel of putative candidate genes identified HMGA2 and PTPRF as IMP3 targets in hCPC. Therefore, HMGA2 and PTPRF are potentially involved in hCPC proliferation/ migration regulation.

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Keywords: human cardiac progenitor cells, Interleukin 1A, IMP3 (IGF2BP3)

Poster: 251

DIRECT CONVERSION OF PORCINE PRIMARY FIBROBLASTS INTO HEPATOCYTE-LIKE CELLS

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The pig is an important model organism for biomedical research, mainly due to their extensive genetic, physiological and anatomical similarities with humans. Until date, direct conversion of somatic cells into hepatocyte-like cells (iHeps) has only been achieved in rodents and human cells. Here, we employed lentiviral vectors to screen a panel of twelve hepatic transcription factors (TF) for their potential to convert porcine fibroblasts into hepatocyte-like cells. We demonstrate for the first time, hepatic conversion of porcine somatic cells by over-expression of CEBPα, FOXA1 and HNF4α2 (3TF-piHeps). Reprogrammed 3TF-piHeps display a hepatocyte-like morphology and show functional characteristics of hepatic cells, including albumin secretion, DiI-AcLDL uptake, storage of lipids and glycogen and activity of cytochrome P450 enzymes CYP1A2 and CYP2C33 (e.g. CYP2C9 in humans). Moreover, we show that markers of mature hepatocytes are highly expressed in 3TF-piHeps, while fibroblastic markers are reduced. We envision piHeps as useful cell sources for future studies on drug metabolism and toxicity as well as in vitro models for investigation of pig-to-human infectious diseases.

Funding Source: Reinhard Koselleck Grant from DFG (Deutsche Forschungsgemeinschaft). HiLF-I grant from MHH (Hannover Medical School)

Keywords: hepatic direct conversion, porcine hepatocyte-like cells, CEBPα; FOXA1; HNF4α2.

Poster: 252

GLUCOSE DURING IN VITRO PANCREATIC BETA CELLS REGENERATION: FRIEND OR FOE?

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The generation of pancreatic beta cells from human pluripotent stem cells can be an alternative source for beta cell replacement therapy. Different protocols for the in vitro generation of beta cells from stem cells have been developed over the years. The in vitro

differentiation process has been performed under high glucose concentration conditions (≥ 20 mM), but its role is unclear. In this study, we investigated the effects of differentiating human induced pluripotent stem cells (hiPSCs) to insulin-producing cells (IPC) under physiological glucose concentration (5.5mM) compare to high glucose (20 mM). The Babk2 hiPSC line was differentiated following a seven-stage protocol in Geltrex-coated cell culture plates. The endocrine-related gens expressions were analyzed via RT-PCR, and proteins were analyzed by immunofluorescent staining followed by quantification analysis with Fiji. Differentiated Stage 5 (pancreatic progenitor stage) to Stage 6 (stem cell-derived beta cell stage) cells in 5.5mM glucose showed a 2.9 times higher expression of NKX6.1 ($P<0.001$) compared to 20mM glucose. GLUGACON, NEUROD1, and MAFA expression were also significantly higher ($P<0.05$). Meanwhile, other endocrine-related genes such as ARX, PDX1, INSULIN, NKX2.2, and SST did not show significant changes to different glucose concentrations. An average of 52% INSULIN-positive cells co-expression with NKX6.1 in 5.5mM glucose at Stage 6 versus 12% co-expression in 20mM glucose ($P<0.001$). 95% of NKX6.1+ cells have co-expression with PDX1 compared to 21% in 20mM glucose ($P<0.001$). ARX and PDX1 co-expression have not been infected. Culturing the cells previously differentiated under 20mM glucose at Stage 7 (beta-cell maturation stage) with 5.5mM glucose showed irreversible loss co-expression of NKX6.1/PDX-1, which might be due to the negative effect of high glucose concentration during Stage 5 and 6. Our results showed that cells differentiated under physiological glucose concentration (5.5mM) express higher endocrine-related genes. The cells differentiated with high glucose concentration induced an irreversible reduction in the co-expression of PDX1/NKX6.1. Therefore, high glucose concentrations might not be beneficial for human iPSCs to IPC differentiation.

Keywords: Human iPSC-derived beta-cell, Endocrine lineage, Differentiation

Poster: 253

NIACIN AND FGF-2 PRETREATMENT REDUCE SENESCENCE INDUCTION IN HUMAN MESENCHYMAL STEM CELLS AFTER SULFUR MUSTARD EXPOSURE

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Chronic senescence in human mesenchymal stem cells (MSCs) was recently identified as novel pathomechanism after exposure to the alkylating chemical warfare agent sulfur mustard (SM). There is no causal therapy available and especially the delayed wound healing, which may result in chronic wounds, is a major obstacle. Due to their longevity and proinflammatory microenvironment, these wounds may be caused by senescent MSCs. Preventing senescence induction after SM exposure might be a possible treatment option. In this study, the growth factor FGF-2, the vitamin niacin, and the poly(ADP-ribose)polymerase inhibitor olaparib were tested as pre- or post-exposure treatment. Human MSCs were isolated from bone marrow of femoral heads and cell identity was verified by their differentiation potential determined by IncuCyte microscopy. Senescence was induced by exposure to 40 µM SM and senescence-associated β-galactosidase (SA-β-gal) was stained up to 21 days later. 5 ng/ml FGF-2, 15 µM niacin, or 10 µM olaparib were added 23 h before as pre- or starting from 1 h after SM as post-exposure treatment, respectively. Non-senescent solvent controls as well as sham-treated controls were performed simultaneously. Osteogenic and adipogenic differentiation of MSCs could be tracked on-line. Video records showed the process in more detail rather than histochemical staining at the endpoint. Pretreatment with FGF-2 and niacin significantly reduced the senescence induction 21 days after SM exposure and did not affect non-senescent controls at the same time. Post-exposure treatment with both substances did not show the same effect. In contrast, olaparib pre- and post-exposure treatment itself significantly increased senescence in non-senescent controls but showed no effect after SM exposure. In conclusion, single dose prophylactic treatment with the growth factor FGF-2 and the vitamin niacin appears sufficient to reduce the induction of chronic senescence in human MSCs. Since senescent MSCs may be unable to fulfil their regenerative role in wound healing, a reduced senescent cell burden already during induction might possibly result in an innovative treatment strategy for SM exposure.

Keywords: mesenchymal stem cells, senescence, wound healing disorder

Poster: 254

CHARACTERIZATION OF A NATURALLY OCCURRING PLURIPOTENT STEM CELL POPULATION ISOLATED FROM HUMAN PERIPHERAL BLOOD

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Regenerative medicine is an ever-growing scientific field that gives new therapeutic approaches in several diseases, frequently utilizing pluripotent stem cells. Embryonic pluripotent stem cells, although the most suitable candidates, come with limitations that include the inadequate availability and the ethical dilemmas in their isolation and use. On the other hand, induced pluripotent stem cells (iPSCs) offer an unlimited supply of autologous cells that could be used without the risk of immune rejection, but have also disadvantages, such as possible carcinogenesis, high cost and excessive manufacturing time. A possible solution is to use donor iPSC cell lines to produce allogeneic "off-the-shelf" tissue matching cell products, but this process, although timesaving, is still relatively expensive and demands immunosuppression. Hypothetically, naturally occurring PSCs found in abundance, with low cost, less manufacturing time, and used autologously, would eliminate the need for immunosuppression. We have discovered and characterized, using immunohistochemical analysis, a population of cells in human peripheral blood that are pluripotent, easy to isolate, and abundant. This discovered population ranges between 1 to 5 million cells per ml of plasma and consists of cells that are relatively small in diameter (<5um). These cells possess a pluripotent identity, as they stain positive for the Kyoto Probe (KP-1) and express all four Yamanaka factors (OCT4, SOX2, cMYC, KLF4), and the embryonic markers Nanog,

CXCR4, SSEA3 and SSEA4. It is plausible to suggest that these easily obtainable, naturally occurring PSCs from peripheral blood may have positive implications in regenerative medicine, as they could fulfil the need for an unlimited supply of autologous pluripotent stem cells without the risk of immune rejection.

Keywords: Small Blood Stem Cells, pluripotent stem cells, Yamanaka factors

Poster: 255

THE EFFECTS OF 5,10 METHYLENETETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISM ON THE DIFFERENTIATION, COLONY FORMATION AND PROLIFERATION OF HUMAN MESENCHYMAL STEM CELLS

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5,10-Methylenetrahydrofolate reducerase (MTHFR) enzyme plays an important role in folate metabolism. C677T polymorphism is the most common mutation in the Turkish population and causes the activity of the enzyme to decrease. This polymorphism, has been associated with many different diseases in the literature. It is also considered as an indicator of bone diseases and triggers the formation of osteoclast, which causes apoptosis in bone marrow derived mesenchymal stem cells (MSC), resulting in a decrease in bone density. Since MSCs are known to be the main source of bone, cartilage and adipose tissue regeneration, it is thought that there may be a link between MTHFR C677T polymorphism and MSC differentiation capacity. For this reason, the effects of MTHFR C677T polymorphism on MSC viability, morphology, physiology and differentiation capacity were investigated. For this purpose, primary human MSCs with wild type (C/C), heterozygote (C/T) and homozygote (T/T) for the MTHFR gene were obtained with ethical permissions. Mutations were detected using RFLP and Sanger sequencing methods from genomic DNA, colonization properties were investigated by CFU-F test and proliferative differences were investigated by MTT test. Adipogenic, osteogenic and chondrogenic differentiation were induced and the results were statistically analyzed using one-way ANOVA with Graphpad Prism. A total of 13 donors were screened and C677T polymorphism was observed to be 46%. There was no difference in the MSC markers and in vitro morphologies of the cells. While there were significant differences between WT and HTZ as a result of the CFU-F test, there were no significant differences in MTT test after 24 and 48 hours. As a result of differentiation tests, it was found that adipogenic differentiation was significantly more in HMZ cells than WT cells in support of the findings in the literature. Osteogenic and chondrogenic differentiation results did not give statistically significant results, but as a result of chondrogenic differentiation, HMZ cells were observed not to respond to directed differentiation cues. As a result of these experiments, adipogenic differentiation was found to be linked to MTHFR genotype, and it was conceived by considering experimental observations that chondrogenic differentiation may be related to it as well.

Funding Source: TUBITAK (Scientific and Technological Research Council of Turkey) project number 119Z521

Keywords: Bone Marrow derived Mesenchymal Stem Cells, MTHFR C677T, differentiation

Poster: 256

INDUCING FAST-SPIKING NEURONS FROM GLIA IN THE POSTNATAL CEREBRAL CORTEX

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Direct lineage reprogramming of resident glia into induced neurons (iNs) is an emerging concept for the remodeling and restoration of diseased circuits. Using developmentally inspired transcription factors, glia-to-neuron conversion has been successfully achieved in vivo. Several neurological and neuropsychiatric disorders have a developmental origin. Thus, the early postnatal cortex may be the substrate of choice for remodeling circuits affected by neurodevelopmental disorders. Here, we aimed at testing whether the proneural transcription factor Ascl1 in combination with Bcl2) can reprogram glia undergoing developmental expansion (i.e., via proliferation in the absence of prior injury) into functional iNs in the early postnatal cortex. For this, we transduced neonatal (P5) proliferating glia with retroviruses encoding the reprogramming factors and explored the electrophysiological properties of these cells in acute brain slices. We found that cells transduced with Ascl1 and Bcl2 acquired membrane properties similar to immature neurons, displaying transient inward currents and fired single action potentials. Intriguingly, forced co-expression of the phospho-deficient variant Ascl1SA6 and Bcl2 resulted in the generation of iNs capable of repetitive action potential firing. At 4 weeks post injection, Ascl1SA6-Bcl2 derived iNs developed fast-spiking (FS) properties characterized by sustained high-frequency firing (>150 Hz) and received excitatory synaptic inputs. Consistent with these electrophysiological hallmarks of FS-interneurons, cells expressed Kv3.1 channels. Taken together, our data show the potential of a phospho-deficient mutant of Ascl1 to induce FS-interneuron specific features from glia in vivo.

Funding Source: This work was supported by grants of the Wellcome Trust, DFG, and BMBF (NEURON ERA-NET ImprovVision) to BB. NM was supported by a fellowship from the Human Frontiers Science Program (HFSP Long-Term Fellowship, LT000646/2015).

Keywords: Reprogramming glia into neurons, Cell differentiation cerebral cortex, Fast-spiking induced neurons

Poster: 257

BECOMING A MOTHER: DYNAMIC SPATIAL AND TEMPORAL RECRUITMENT OF ADULT NEURAL STEM CELLS DURING PREGNANCY

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Neural stem cells (NSCs) reside in specialized niches in the adult mammalian brain. The largest site of adult neurogenesis in the mouse is the ventricular-subventricular stem cell niche, which extends along the brain lateral ventricles. V-SVZ NSCs give rise to different subtypes of olfactory bulb interneurons as well as some glia throughout life. Adult NSCs are not a uniform

population. Instead they comprise a mosaic of cells with unique molecular identities and differentiation fates, depending on their spatial location in the niche. Moreover, NSCs can be found in both quiescent and actively proliferating states in vivo. The external signals regulating the balance between stem cell activation and dormancy, as well as the factors controlling regionally distinct NSCs, are still largely unknown. Here, we show that pregnancy activates specific stem cell subpopulations residing in spatially distinct domains of the V-SVZ, with different temporal dynamics. The recruitment of 'pregnancy-related' NSCs is transient, and results in the generation of waves of different subtypes of newborn olfactory bulb interneurons that in turn mature at key physiological periods of motherhood, such as birth or perinatal care. A subset of these new cells persists in the mother's brain as an imprint of previous pregnancy experience, whereas others are specifically culled. Interestingly, oligodendrocyte progenitors also increased in the olfactory bulb concomitant with the increase of neurons. Taken together, our results demonstrate pregnancy as a physiological state that recruits distinct pools of adult NSCs for the generation of specific cell types in preparation of the female brain for motherhood.

Keywords: adult neurogenesis, stem cell heterogeneity, pregnancy

Poster: 258

THE MIR-17~92 CLUSTER: A REGULATOR OF FATE DECISIONS IN THE ADULT V-SVZ NEURAL STEM CELL NICHE

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In the adult mammalian brain, the ventricular-subventricular zone (V-SVZ) generates neurons and glia throughout life. In this germinal niche, neural stem cells (NSCs) coexist in quiescent and activated states, and predominately contribute progeny through transit-amplifying cells (TACs). miRNAs have been implicated in stem cell self-renewal and differentiation. By performing miRNA profiling of FACS-purified quiescent and activated adult NSCs, we identified the miR-17~92 cluster as highly upregulated in activated stem cells in comparison to their quiescent counterparts. We validated this finding using qPCR and in situ hybridization. Conditional deletion of miR-17~92 in FACS-purified adult NSCs reduced NSC activation, proliferation, and self-renewal in vitro. Similarly, miR-17~92 deletion in GFAP+ NSCs decreased NSC activation and neurogenesis in vivo. Unexpectedly, we also saw an expansion of OLIG2+ TACs at the expense of DLX2+ TACs. This led to increased oligodendrogenesis in the V-SVZ and corpus callosum. Together, these data uncover new functions of the miR-17~92 cluster in cell fate decisions and oligodendrogenesis in the adult V-SVZ.

Keywords: Neural stem cells, cell fate, oligodendrogenesis

Poster: 259

THE ROLE OF LIPID METABOLISM IN HUMAN BRAIN DEVELOPMENT

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Neural stem cells (NSCs) give rise to the entire brain and even continue to form new neurons throughout life. Understanding what regulates NSC behavior is thus important both for development and for adulthood. Recently, lipid metabolism, specifically the build-up and break-down of lipids, has been shown to have an important role in the regulation of NSC quiescence, proliferation and integration of their progeny in the mouse brain. However, whether lipid metabolism plays a similar role in the regulation of human NSCs during brain development remains poorly understood. We are using human induced pluripotent stem cells (iPSC)-derived NSCs and cerebral brain organoids to study the influence of lipid metabolism pathways with pharmacological and gene silencing approaches. Here, we show first results indicating that lipid metabolism is also important for human NSCs.

Keywords: Neural stem cell, Lipid metabolism, Development

Poster: 260

METABOLIC PROFILING OF NEURAL STEM/ PROGENITOR CELLS REVEALS REGIONAL IDENTITY

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Cellular metabolism has emerged as a potent regulator of proliferation and fate decision in neural stem/progenitor cells (NSPCs). In the adult mouse brain, there are at least two neurogenic niches, namely the subventricular zone (SVZ) of the lateral ventricle and the sub-granular zone (SGZ) of the dentate gyrus (DG) in the hippocampus. Both niches contain NSPCs which give rise to newborn neurons, however, while the neurons in the DG stay close to their place of birth, the ones born in the SVZ have to migrate a long distance to the olfactory bulb. Whether the metabolic regulation of NSPCs from these two niches is similar has not been addressed in detail. Furthermore, despite evidences of sex differences in neurogenesis, it is not known if NSPC metabolism differs between males and females. In order to address these questions, we extracted NSPCs from the SVZ and DG of individual adult male and female mice and expanded them in vitro for several weeks. We subsequently performed gene expression analyses together with an open chromatin assay (ATAC-seq), and assessed the metabolic prolife of these NSPCs by untargeted metabolomics. Our preliminary data show that NSPCs from the DG and SVZ keep a distinct metabolic profile, despite prolonged time of growth in vitro, suggesting that regional identity is also kept on a metabolic level. Furthermore, a few metabolites seem to be sex-specifically regulated, providing interesting new avenues to explore to better understand sex differences in neurogenesis.

Keywords: neural stem cells, Metabolic profiling, Regional identity

Poster: 261

A NOVEL MOUSE MODEL TO STUDY LIPID DROPLETS IN NEURAL STEM/PROGENITOR CELLS AND THEIR NICHE

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Over the recent years several studies have highlighted lipid metabolism as an important regulator of neurogenesis. It has been shown in mice that neural stem/progenitor cells (NSPCs) rely on de novo lipogenesis for proliferation, and that fatty acid oxidation (FAO) is important for quiescence. De novo lipogenesis and FAO are tightly linked, and shifting the balance between these two pathways has consequences for the activity and function of NSPCs. Lipid droplets (LDs) are the lipid storing organelles in cells and have a key function for lipid metabolism: LDs can store newly synthetized lipids from de novo lipogenesis and at the same time be an important lipid reservoir for FAO. Thus, it is of great interest to investigate the role of LDs in NSPCs and the neurogenic niches. We have created a novel LD reporter mouse by endogenously tagging the LD coat protein Perilipin-2 (Plin2) with tdTomato, using CRISPR/Cas9. This endogenous expression of tdTomato-Plin2 allows for direct visualization of LDs in vitro and in vivo in fixed and alive cells and in tissues. We are using this novel reporter mouse to study the distribution of LDs in NSPCs and in the neurogenic niches of the adult and developing mouse brain. We are also studying the dynamics of LDs using live imaging and investigating what happens to the LDs in the mouse brain after a short-term high-fat diet. In this poster, we will present our latest results obtained with this LD reporter mouse model.

Funding Source: Swiss National Science foundation (#31003A_175570)

Keywords: Neurogenesis, Lipid metabolism, Lipid droplets

Poster: 262

THE FATE OF SUSD2+ ENDOMETRIAL MESENCHYMAL STEM CELLS DURING IN-VITRO DECIDUALIZATION

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Endometrial regeneration in premenopausal women is maintained by the so-called endometrial mesenchymal stem cells (eMSC) upon exposure to estrogen and progesterone. eMSC located in the perivascular niche of endometrium express so-called Sushi Domaine Containing 2 (SUSD2). The role of these eMSCs in pregnancy and post-partum regeneration is unknown. Our aim was to determine the effect of progesterone on the stemness of the SUSD2+ eMSC isolated from non-pregnant uterine samples. A secondary objective was to characterize the functional capacity of SUSD2+ eMSC isolated from the uterus at term pregnancy and to compare it with the capacity of those isolated from non-pregnant uterine samples. Firstly, SUSD2+ eMSC from non-pregnant uterine samples were treated with progesterone to induce decidualization. Changes in membrane markers and gene expression profile of several genes involved in the decidualization were determined. Results suggest that eMSC could play an important role in the course of embryo implantation. Furthermore, the abundance of SUD2+ eMSC was determined with immunohistochemistry in the non-pregnant and both 1st trimester and term pregnancy uterine samples. Histological analysis revealed a statistically significant lower abundance of eMSC in 1st trimester and term samples compared to non-pregnant samples. To characterize the functional capacity SUSD2+ cells were differentiated and CFU assay was performed. The capacity to differentiate into mesenchymal lineages and form colonies did not differ significantly between the cells isolated from non-pregnant and pregnant uterine samples. Further studies with progesterone will contribute to a better understanding of SUSD2+ eMSC's fate during decidualization and regeneration.

Funding Source: Funded by the FWF der Wissenschaftsfonds.

Keywords: endometrial stem cells, decidualization, pregnancy

12:00 - 13:00 EDT

POSTER SESSION 3

NEW TECHNOLOGIES

Poster: 301

CHARACTERIZATION OF PRIMARY AND IMMORTALIZED HUMAN ADIPOSE STEM CELLS CULTURED IN A NOVEL SERUM-FREE XENO-FREE MEDIA

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Adipose stem cells (ASCs) are a type of mesenchymal stem cells that may be isolated in abundance from adipose tissue. However, ASCs have a limited lifespan in vitro and typical culture conditions involve the use of 2-10% serum, human or animal, to enhance cell attachment and as a source of growth factors. Since these cells release secretory factors with potential therapeutic effects, it is crucial to develop stable ASC lines and to eliminate serum supplements from the culture media to improve culture stability, secretory factor scalability and to prevent the risk of adverse immune reactions when used therapeutically. Here, we have developed immortalized ASC lines and formulated a superior, unique, completely defined serum-free and xeno-free media (SFM) that supports the growth of these cells in vitro and which may be utilized for clinical applications. Primary ASCs were isolated from anonymized biospecimens, immortalization performed by transduction with SV40 and hTERT genes, and, both primary and immortalized cells cultured in vitro with a novel Serum-Free Xeno-Free Media developed in our laboratory.

Morphology, viability, proliferation rates, differentiation potentials, and cytokine profiles were evaluated in both groups of cells. Co-transductions successfully immortalized ASCs resulting in lines with characteristic ASC morphologies marker expression, and similar differentiation potentials to mother lines and population doubling levels of greater than 60 thus far. Our complete SFM supported the growth and proliferation of both immortalized ASCs and primary ASCs (up to passage 14 in vitro) while maintaining their mesenchymal stem cell characteristics and stemness. These cells were successfully cultured in monolayers and three-dimensional scaffolds, were able to form structures like embryoid bodies, and, differentiated to adipocytes, chondrocytes and osteocytes. The immortalized cells also exhibited a secretory profile similar to that of primary ASCs but with greater consistency. Hence, these ASCs and their secretory factors may be safely used for downstream therapeutic or regenerative applications without the risk of viral transmission or adverse immunological reactions.

Keywords: Immortalized, Serum-free, Novel

Poster: 302

ROBUST AND OPTIMIZED TESR MEDIA AND WORKFLOWS FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURE

Jervis, Eric, Eaves, Allen, Hukazalie, Kyle, Louis, Sharon A., Markwick, Karen, Walsh, Kylan

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Suspension culture enables scale-up of human pluripotent stem cell (hPSC) manufacturing. However, the use of media optimized for 2D adherent culture can result in low volumetric productivity and laborious workflows when applied to 3D culture. To overcome these limitations, we have developed a portfolio of TeSR™-based media for suspension culture of hPSCs as aggregates consisting of a seed medium and a fed-batch feed supplement. Media was optimized by iteratively modifying the formulation to maintain target nutrient levels and maximize growth rates and cell quality during scale-up. The 3D portfolio consists of mTeSR™ 3D, based on mTeSR™1, TeSR™-E8™ 3D, a low protein formulation based on TeSR™-E8™, and a newly developed animal component free formulation TeSR™-AOF 3D. Cell expansion experiments were conducted using human embryonic (H1, H7, and H9) and induced pluripotent (WLS-1C, R038, STiPS-F016) stem cell lines for up to ten passages. Aggregates were passaged by dissociation into small clumps using Gentle Cell Dissociation Reagent then re-seeded into fresh medium. Cultures were passaged with alternating 3 / 4 day cycles, with fed batch feeding to minimize culture perturbation, and a 50% media exchange on day 3 of a 4 day cycle. This feeding strategy minimizes labour requirements, does not lead to metabolite accumulation, and maximizes cell expansion and quality. Protocols are highly robust to enable routine hPSC expansion in suspension across multiple culture volumes; 2 mL in 6 well plates, 10-60 mL in orbital shaker flasks, 60-500 mL with the PBS Biotech Bioreactor system. Seeding densities and mixing rates were optimized at each scale-up stage for each cell line. Overall all TeSR™-3D media formulations and protocols routinely enable greater than 1.3 to 1.8 fold expansion per day (cell line dependent), >80% viability, >90% expression of OCT4 and TRA-1-60, the capacity to differentiate to three germ layers, and normal karyotypes. Analysis across cell lines (n=6) has revealed that different cell lines may require specific formulations and mixing rates for optimal growth in suspension. The TeSR™ fed-batch 3D portfolio of products enables users to select the appropriate media for the manufacture of hPSCs as aggregates in suspension cultures, with an efficient workflow and improved volumetric productivity.

Keywords: Cell Manufacturing, PSC Culture Scale-up, 3D Suspension Culture

Poster: 303

PAIRING 3D PLURIPOTENT STEM CELL SUSPENSION CULTURE WITH BIOREACTOR SYSTEMS TO ENABLE EFFICIENT SCALE-UP AND CRYOPRESERVATION OF LARGE NUMBERS OF HIGH-QUALITY CELLS

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As the need for pluripotent stem cells (PSCs) continues to rise, a key bottleneck is the generation of sufficient numbers of high-quality PSCs. To achieve the large numbers of cells necessary for therapeutic and screening applications, three-dimensional (3D) suspension cultures offers key advantages over two-dimensional (2D) adherent cultures. In particular, the overall cost and reduced consumption of plastics makes suspension cultures more desirable for scale-up. However, existing solutions and protocols are often unable to utilize the full potential suspension cultures can yield. With the recent launch of our new 3D suspension culture medium – Gibco™ StemScale™ PSC Suspension Medium – we offer the ability for users to rapidly and efficiently scale-up to large numbers of cells. StemScale Medium promotes the self-aggregation of singularized PSCs into spheroids. These spheroids exhibit robust expansion over a (typical) 4 – 5 day culture period, all while maintaining highly viable and pluripotent cells. Cultures are seeded at a density of 150,000 cells/mL and fed with periodic 50% medium replacement. This versatile system supports efficient scale-up in a variety of suspension culture vessel sizes, from small-scale (well plates and shake flasks on an orbital shaker platform) to large-scale (3L bioreactors). For example, within 3 passages, users can scale-up from 6-well plates to 3L bioreactors, generating cell numbers in the 1-1.5E9 PSCs/ml range. We observe that expansion in small-scale orbital shaker platform cultures is comparable to expansion in large-scale 3L bioreactor systems, enabling users to achieve similar growth rates across a range of culture vessels and formats. Importantly, we also demonstrate that the cells obtained from spheroids grown in StemScale Medium are readily dissociated and capable of being cryopreserved, with no noticeable impact on performance after recovery from thaw. Ultimately, this allows users the flexibility to: use the expanded spheroids for directed differentiation (in 3D), dissociate the PSC spheroids and immediately use the cells for downstream applications or characterization (in 2D), reseed the cells back into suspension cultures for additional passages, or cryopreserve the singularized cells for use at a later date.

Keywords: Spheroid, Bioreactor, Suspension Culture

Poster: 304

UTILIZING MACHINE LEARNING TECHNIQUES TO FABRICATE SERUM FREE MEDIA FOR SEAFOOD INDUSTRY

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The booming population and recent emergence of COVID-19 have made issues like food insecurity and shortages extremely problematic. Seafood is a vital food-based product that requires sustainability because of its high global consumption rate and excellent nutritional profile. One of the most sustainable future seafood options is cultivated meat that can provide authentic in-vitro meat without slaughtering and has potential environmental and economic advantages. Despite the advantages and current investment of over $350 million, cultivated meat's implementation is hampered at the industrial level due to current cell culture media requirements. Fetal Bovine Serum is the most commonly used component of cell culture media that aids in cell proliferation, but it has many disadvantages, such as low reproducibility and high cost. Thus, employing serum-free media like chemically defined media (CDM) is an excellent serum-free media option. However, its development is an arduous task due to time-consuming experimentations and high-cost. Machine learning techniques (MLT) have set a pioneering milestone in numerous media optimization problems. MLT's such as Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) have proven to be ideal tools to optimize media for various cells. Machine learning reduces the overall effort required for media optimization in terms of experimental number, cost, and time and creates predictive models used as gold standards for future optimizations. The most effective way to gain MLT techniques' benefits is to use robust statistical tools like RSM to generate an appropriate minimum number of experiments and feed the data gathered from them into an ANN for predictive modeling. This effective combination of two powerful MLT's has the potential to give us the best optimization in minimum time with future predictive models. Using AI-based optimizations will be our work's future goal, targeting future media optimizations for new seafood-based cell lines.

Funding Source: Good Food Institute (G.F.I.)

Keywords: Cultured meat, Response Surface Methodology (RSM), Artificial Intelligence (AI)

Poster: 305

AN ANIMAL FREE FULLY DEFINED WORKFLOW FOR HUMAN INDUCED PLURIPOTENT STEM CELL RESEARCH

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Human induced pluripotent stem cells (iPSCs) are critical for creating ex vivo models for studying developmental processes, disease mechanisms and performing drug discovery. IPSC also serve as the basic raw material for multiple cell therapeutics in clinical development and trials. However, many iPSC research workflows involve undefined culture surfaces, with lot-to-lot variability, and cell medium with animal components that may introduce confounding factors. These undefined factors may alter cellular identity and function during cell expansion and differentiation and severely limit a study's reproducibility. From both a scientific and manufacturing point of view, it is critical to overcome these obstacles by creating an animal free and fully defined workflow that will ensure validity and reproducibility of results and easier compliance with clinical cGMP standards. To create this animal free workflow, we devised and screened multiple mutated vitronectin fragments for their capacity to support iPSC attachment and proliferation over multiple passages. We characterized both colony health, cell identity, and growth rate for different mutants. Additionally, we created an animal free iPSC media that supported the proliferation and growth of six different iPSC lines for over forty passages. Human IPSC in this media showed proper colony formation with healthy morphology throughout and expressed positive markers of stemness such as Oct3/4, SSEA-4 with no expression of differentiation markers such as SSEA-1 as assayed with flow cytometry and ICC. No karyotype abnormality with this media was detected using G-band analysis. The cells also successfully differentiated to multiple cell types including germ layers and cardiomyocytes. Finally, we created an animal free neuronal differentiation process and successfully obtained neurons using the aforementioned vitronectin fragment, iPSC media and animal-free neuronal supplements. These results demonstrate the feasibility of a standardized fully defined, animal free, and reproducible process for iPSCs research and clinical translation.

Keywords: induced pluripotent stem cell, Animal Free Defined, vitronectin

Poster: 306

SCALABLE MANUFACTURING OF HUMAN MESENCHYMAL STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS IN VERTICAL-WHEEL BIOREACTORS

Borys, Breanna[1], Rosello, Francisco[1], Dang, Tiffany[2], Gray, Kelly[1], Kanwar, Shivek[2], Colter, James[2], Worden, Hannah[1], Ham, Dong-sik[3], Song, Sun-uk[4], Lee, Brian[5], Kallos, Michael[2], Jung, Sunghoon[1]

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Human-derived mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) have amazing potential for the treatment of serious diseases. In order to succeed as allogeneic cell therapy products, a consistent and scalable cell culture process using suspension-based, single-use bioreactors is required to meet clinical and commercial production demands. However, the hydrodynamic characteristics of conventional stirred-tank bioreactors (STRs) can present scalability problems for cells such as MSCs grown on microcarriers or iPSCs grown as aggregates. Specifically, the fluid shear stress and heterogeneous mixing conditions caused by rapidly spinning horizontal impellers in STRs can negatively affect growth and quality of shear-sensitive cells. This presentation provides examples of how the unique hydrodynamic conditions of Vertical-Wheel (VW) bioreactors – low shear stress and homogeneous energy dissipation rates – promote robust and scalable manufacturing

of MSCs and iPSCs. In one example, a highly consistent process was developed for clinical-scale production of MSCs grown on microcarriers, by identifying and optimizing key process variables for cell growth and harvest. Target yield of 20 billion MSCs (within 14 days starting from 2 million cryopreserved cells) and high cell quality were consistently achieved at 15 L scale, and also in scale-down model processes involving 0.5L and 3L bioreactors. In the other example, iPSCs formed uniformly-sized, spherical aggregates and achieved target cell densities across various scales of bioreactors, which is a critical requirement for efficient differentiation into target therapeutic cells. In summary, the distinctive hydrodynamic conditions of VW bioreactors facilitate reproducible and scalable production of cell therapy products such as MSCs and iPSCs.

Keywords: Allogeneic cell therapy manufacturing, Mesenchymal stem cells and induced pluripotent stem cells, Scalable Vertical-Wheel bioreactor

Poster: 307

A HUMAN STEM CELL-BASED HIGH-THROUGHPUT PLATFORM WITH ARTIFICIAL INTELLIGENCE TECHNOLOGY TO SCREEN FOR DEVELOPMENTAL TOXICANTS

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Environmental factor-induced birth defects raise the risk for lifelong disabilities to those who survive and increases 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. In this study, we developed a human stem cell (hECs)-based high-throughput platform with artificial intelligence technology to screen for developmental toxicants. Embryoid bodies (EBs) generated from hECs were utilized as the model as their formation recapitulates many 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 on both human and experimental animals. The expression change of 20 hallmark genes in embryogenesis was subjected to machine learning with 10 different algorithms. With feature selection, the Random Forest-based classification model showed a good accuracy (mean:53%) to categorize the 35 chemicals correctly into four different risk levels (none, minimal, moderate, and high), forming the first part of the prediction system. The second part of the system is based on the chemical-elicited structural alterations in EBs, captured by high-content imaging with immunostaining with markers (TUJ1, SMA, and SOX17) for each germ layer (Ectoderm, Mesoderm, and Endoderm). A highly accurate (mean: 81%) prediction model backend by the ResNET deep learning was yielded through the training with 4203 images of EBs from each exposure. To further 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 largely 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 33.9% toxicity similarity with Etretinate, a identified teratogen from the same family. Together, these results present a promising capability of our screening platform in identifying human developmental toxicants and understanding their etiology.

Keywords: Human embryonic stem cells, Organoid, Artificial intelligence

Poster: 308

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

Hong, Hyen Jong, Tristan, Carlos A., Simeonov, Anton, Singeç, Ilyas

DPI, NIH\NCATS, Rockville, MD, USA

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

Keywords: human pluripotent stem cell, Gene editing, CRISPR

Poster: 309

CLONER2 FACILITATES EFFICIENT CLONING OF HUMAN PLURIPOTENT STEM CELLS

Hirst, Adam, Lim, Darielle J., Eaves, Allen C., Louis, Sharon A., Hunter, Arwen L.

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Derivation of clonal human pluripotent stem cell (hPSC) lines is a major hurdle in several hPSC workflows including clonal isolation of rare cell populations following CRISPR gene-editing. To overcome this hurdle, we previously developed CloneR™, a single-cell cloning supplement that has been widely adopted by researchers due to significant increases in cloning efficiency

over conventional methods. To further support the field we have now developed CloneR™2, an improved supplement to allow rapid and higher efficiency derivation of clonal hPSC lines. Specifically, eight hPSC lines seeded in mTeSR™1 supplemented with CloneR™2 at low-density (25 cell/cm2) on Vitronectin XF™ coated plates, displayed on average of 2.4-fold increase in cloning efficiency over CloneR™ controls with an average cloning efficiency of 28.8 ± 5.6 (mean ± SD, n=≥7 per cell line). CloneR™2 displayed an average increase in cloning efficiency of 6.4-fold over CloneR™ controls in six hPSC lines plated using single-cell deposition (1 cell/well), with an average cloning efficiency of 18.5% ± 4.2 (mean ± SD n=≥4 per cell line). Colonies derived in CloneR™2 display on average a 2.3-fold increase in colony size eight days following plating, allowing for earlier clonal selection. CloneR™2 has been tested with a range of media and matrix combinations including mTeSR™Plus on Corning®Matrigel® and TeSR™-AOF on Vitronectin XF™, with hPSC lines displaying an average cloning efficiency of 37.6% ± 4.8 and 32.4% ± 11.0 (mean ± SD n=6) respectively. Six randomly selected clones from five hPSC lines derived in CloneR™2 were screened using the hPSC Genetic Analysis Kit. All clones tested negative for aneuploidy at eight recurrently affected chromosomal regions in hPSCs (n=30). Cloned hPSC lines also retain their pluripotent potential as assessed using the STEMdiff™ Trilineage Differentiation Kit. Clones from 4 different hPSC lines displayed efficient differentiation towards ectoderm (PAX6/NESTIN, >89.4%, n=4), mesoderm (NCAM/T, >88.7%, n=4) and endoderm (CXCR4/SOX17, >69.2%, n=4) with all clones demonstrating equivalent differentiation potential to their parental hPSC line (≥90%, n=4). In summary, CloneR™2 is an improved single-cell cloning supplement designed to facilitate gene-editing and other workflows requiring clonal hPSC lines.

Keywords: CRISPR, Cloning, gene-editing

Poster: 310

TRANSCRIPTOMIC COMPARISON OF TWO METHODS OF HEPATOCYTE DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Directed differentiation of human induced pluripotent stem cells (iPSCs) into hepatocytes could afford unlimited supply of liver cells, and therefore holds great promise for regenerative medicine, disease modeling, drug screening, and toxicological studies. A variety of methods have been established during the past decade for the differentiation of hepatocyte-like cells (HLCs) from iPSCs using various growth factors. 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 compared two well-known hepatocyte differentiation methods by transcriptomic characterization of the resultant HLCs at early maturation (day 17) and late maturation (day 24) stages. For method 1, only 173 genes changed expression from day 17 to day 24; in comparison, 942 genes altered expression between day 17 and day 24 for method 2. These results suggest that extended cell culture after day 17 further promoted HLC maturation in method 2 but less so in method 1. Comparison of the HLCs at day 21 from both methods to primary human hepatocytes (PHHs) revealed vast differences between HLCs and PHHs, although HLCs generated from method 2 were slightly better than those from method 1 (12,537 vs 14,595 DEGs). Next, we focused the analysis on the 1,481 DEGs identified between HLCs of the two methods at day 21. Functional analysis of the DEGs revealed 290 gene ontology (GO) terms in biological process associated with these genes, which were further categorized into 34 functional classes, with metabolism, development, cell communication, signal transduction, and cell differentiation on top of the list. Furthermore, 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. Therefore, the results of the current study provide molecular basis for the differences observed between the two differentiation methods, offer new insights into gene regulations in hepatogenesis in vitro, and suggest ways to further improve hepatocyte differentiation in order to obtain more mature HLCs for biomedical applications.

Keywords: hepatocyte differentiation, induced pluripotent stem cells, transcriptomics

Poster: 312

AUTOMATED HIGH-THROUGHPUT CHARACTERIZATION OF SMALL MOLECULE COMPOUNDS USING HUMAN PLURIPOTENT STEM CELLS

Ryu, Seungmi[1], Braisted, John[1], Chu, Pei-Hsuan[1], Huang, Ruili[1], Itkin, Zina[1], Malley, Claire[2], Michael, Sam[1], Ormanoglu, Pinar[1], Shinn, Paul[1], Simeonov, Anton[1], Singec, Ilyas[1], Tristan, Carlos[1]

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To perform drug screening in hPSCs and differentiated progeny, it is important to establish standardized and scalable methods that can be automated and used for high-throughput projects. Here, we describe a quantitative high throughput screening (qHTS) protocol that combines various automated methods for hPSC-based screening. The use of automation enables efficient screening of multiple compounds across different concentrations. To demonstrate utility of this qHTS approach, we tested the effects of 12 small molecule compounds, commonly used in the stem cell field, focusing on cell viability and cellular stress using different assays and endpoints. Prior to screening, we also defined the ideal experimental conditions for screening in 384-well plates such as selecting the appropriate coating substrate, plate source, cell density, and solvent concentration. Taken together, the integrated use of different high-throughput assays provides comprehensive information on how widely used small molecules affect cell viability and cell quality encompassing systematic measures of cell membrane permeability/integrity, mitochondrial membrane potential, and ATP production.

Keywords: quantitative high throughput screening, small molecules, viability

Poster: 313

ESTABLISHMENT OF A PLATFORM FOR EX VIVO GENE EDITING IN MULTIPOTENT MOUSE HEMATOPOIETIC STEM CELLS

Hsu, Ian, Wilkinson, Adam, Dever, Daniel, Baik, Ron, Camarena, Joab, Charlesworth, Carsten, Morita, Chika, Nakauchi, Hiromitsu, Porteus, Matthew

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CRISPR/Cas9 gene editing technologies in combination with autologous hematopoietic stem cell transplantation (HSCT) have the potential to cure a wide range of genetically-inherited hematopoietic malignancies. However, explorations into ex vivo gene correction of HSCs intended for autologous HSCT have been limited by current difficulties in growing hematopoietic stem cells (HSCs) ex vivo. In this study, we demonstrate that murine functional long-term HSCs capable of generating all peripheral blood lineages can be gene-targeted at both the Rosa26 safe harbor locus and the beta-globin (HBB) gene locus using Cas9-AAV6-editing technology. Using recently published methods of expanding mouse HSCs ex vivo, we were able to boost the frequency of homology-directed repair (HDR) by ~2.5 fold through extended ex vivo expansion cultures, which generated stable multilineage donor chimerism in serial transplantation assays. Additionally, we were able to achieve stable in vivo production of hemoglobin-A in a HBB sickle cell disease mouse model following autologous transplantation of ex vivo gene-edited HSCs containing HDR-corrected alleles at curative levels (>20%), as measured through droplet digital PCR (ddPCR). In summary, this study establishes a useful platform for genetically modifying multipotent mouse HSCs ex vivo and also identifies areas for improving gene editing in functional HSCs in future.

Funding Source: CIRM, NIH, Leukemia and Lymphoma Society, Amon Carter Foundation, Laurie Kraus Lacob Faculty Scholar Award

Keywords: hematopoietic stem cell, genome editing, hematopoietic stem cell transplant

Poster: 314

CRISPR-CAS9 MATERIALS FOR HUMAN IPSC-BASED CELL THERAPY RESEARCH

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As cell therapies advance, CRISPR-Cas9 has been identified as a key tool for engineering cells due to the simplicity and effectiveness. To be suitable as an ancillary material for cell therapy applications, these gene editing tools require new manufacturing and quality control processes to meet the safety and regulatory standards. Our development team has established a new method to produce a recombinant Cas9 protein that is suitable as an ancillary material for cell and gene therapy applications. Here we demonstrate the use of this newly manufactured Cas9 protein under our Cell Therapy Systems "CTS" labeling – CTS TrueCut Cas9 Protein – as benchmarked against our catalog Cas9 product in both primary T cells as well as our iPSC line. Additionally, our extensive battery of quality control release tests will be shared. The CTS TrueCut Cas9 Protein will advance the use of the CRISPR-Cas9 technology as a preferred non-viral tool for engineering iPSCs in cell therapy development.

Keywords: CRISPR-Cas9, cell therapy, GMP

Poster: 315

EVALUATION OF THE EFFECTS OF SEVERAL SMALL MOLECULES IN THE DEVELOPMENT OF EFFICIENT AND PRECISE METHODS FOR CRISPR/CAS9 EDITING OF HUMAN CELLS

Richards, Colleen[1], MacArthur, Chad[2], Kandell, Jennifer[2], Lakshmipathy, Uma[2]

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The use of CRISPR/Cas9 technology for precise gene-editing applications in human cells has advanced greatly in recent years, and it is now possible to perform genetic editing at specific loci in the human genome. However, these edits require careful follow-up screening and selection of cells to ensure that any cells used for research or clinical applications are free of off-target edits and unwanted mutations. When successfully edited, these cells can be used to study genetic diseases, test potential pharmaceutical candidates, create genetically modified cells for autologous transplant, and more. Therefore, the development of methods to create CRISPR/Cas9 edited human cells with high efficiency and few off-target effects is essential for the expansion of research and clinical applications of this technology. Here we present an analysis of the effects of 6 small molecules on editing efficiency in human cells. These small molecules were selected based on their ability to target pathways related to cell proliferation and survival and regulate DNA repair after double-stranded breaks. The use of these small molecules, alongside optimized editing materials and conditions, may improve both knock-out and knock-in efficiency of a sequence of interest at a specific locus in human cells. Preliminary results show an increase in knock-in efficiency after incorporation of several of these small molecules into the electroporation buffer.

Keywords: Gene editing, CRISPR, CRISPR/Cas9, Electroporation

Poster: 316

A UNIVERSAL QPCR STANDARD FOR DETERMINATION OF IDENTITY, SAFETY, AND POTENCY OF LENTI VIRUS

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Ex vivo modification of cells involving lenti viruses requires high quality functional virus that can transduce cells at high efficiency without impacting cell viability or growth. In addition to robust upstream and downstream lentivirus production processes, reliable methods for characterizing the virus are critical to determine dose (vector genome titer), identity (presence of transgene), purity (residual impurities such as plasmid DNA and process impurities such as residual host DNA and protein), and

safety (replication competent virus and adventitious agents). Additionally, transduced cells are tested for efficacy and safety by determining the average number of copies per cell using a vector copy number assay. Commonly used to determine copy number variation, ddPCR offers precise detection of small changes with high contaminant sample background but is limited by independent data collection and sample preparation time. Alternatively, qPCR is advantageous because it provides a broad range in expression level detection and is often used to determine viral titer and vector copy number. However, the lack of clear standards makes accurate quantification and standardization of the resulting assays challenging. Here, we propose a universal standard that has ~150bp portions of critical targets comprising of viral components, plasmid antibiotic selection, and two house-keeping genes each separated by 100bp spacers with a Multisite Gateway region to insert targets for a gene of interest. The entire region is designed with multiple unique restriction sites that allows for generating linearized standards and standard curves using validated TaqMan qPCR primers for the individual targets. As proof of concept, functional titer of multiple preparations of lentivirus encoding the second generation (CD3ζ and 4-1BB) anti-CD19 CAR was determined using flow cytometry and qPCR methods. Results indicate the titers determined using the two methods are comparable thus supporting qPCR reliability and accurate target quantification. The universal standard was also used to determine vector copy number in CAR-T cells generated using multiple lentivirus at various MOIs. In conclusion, such a universal standard facilitates implementation of robust quality and safety assays critical to GMP production of lenti viruses.

Keywords: Lentivirus, qPCR, CAR T cell

Poster: 317

TECHNOLOGY-ENABLED DRUG DISCOVERY PLATFORM USING HUMAN IPS CELL-DERIVED NEURONAL MODELS, OPTOGENETICS AND MACHINE LEARNING

Williams, Luis A.[1], Fink, James[1], Joshi, Vaibhav[1], Borja, Gabriel[2], Ryan, Steven[3], Delaney-Busch, Nathaniel[3], Pichler, Monika[3], Lu, Yang[4], Gerber, David[4], McManus, Owen[5], Dempsey, Graham T.[5]

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Productivity in CNS therapeutic development remains low given the complexity of the target tissues and lack of scalable models. While progress has been made in advancing human iPS cell-derived (hiPSC-) neurons for disease modeling, generation of robust, reproducible reagents and characterization with high-throughput methods remain a significant challenge. Here, we present a novel platform that integrates three technology axes: i) scalable hiPSC-neuronal models, ii) high-throughput all-optical electrophysiology methods and iii) machine learning-based analytics. Billions of hiPSC-derived neurons are produced and cryopreserved for use in multi-well plate screening formats. Neurons are assayed using Optopatch, which allows simultaneous optical stimulation and recording of neuronal action potentials (APs) using genetically encoded proteins CheRiff (for AP stimulation) and the voltage-sensing protein QuasAr. Optopatch maintains the rich information content of manual patch clamp but with >10,000-fold higher throughput. The fully automated system is compatible with 96 and 384-well plates and can record from 200 neurons/movie and >600,000 neurons/day across ~3000 wells/day, enabling HTS campaigns. Neuronal excitability is assayed by measuring neuronal spike timing and AP shape properties in response to a variety of optogenetic stimuli. Machine learning of Optopatch data sets across thousands of hiPSC-neurons and hundreds of electrophysiological parameters enables identification of robust disease-associated phenotypes. Using Q-State's platform in a human iPSC model of epilepsy comprising 15 different isogenic iPSC clones, 2 disease-causing variants in the KCNQ2 gene (loss-of function KCNQ2-T274M and gain-of-function KCNQ2-R201C), and Optopatch data collected from >70,000 individual neurons, we identified >200 electrophysiological features associated with the expression of mutant KCNQ2, with a majority of these features showing opposing effects in the two variants relative to control. In addition to epilepsy, we have utilized this platform for pain and other neurological disorders and have leveraged our neuronal phenotypes in the context of drug discovery across diverse therapeutic modalities such as small molecules, antisense oligonucleotides and gene therapies.

Keywords: All-optical electrophysiology, iPSC- neurons, Optogenetics

Poster: 318

MICROFLUIDIC CELL SQUEEZING ENABLES THE IN VITRO GENERATION OF INDUCED NEURONS FROM HUMAN PLURIPOTENT STEM CELLS THROUGH NON-VIRAL TRANSCRIPTION FACTOR DELIVERY

Wu, Chun-I[1], Gilbert, Jonathan[1], Tadin-Strapps, Marija[2], Sharei, Armon[3], Bridgen, Devin T.[1]

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Cell replacement therapies are a promising treatment for a variety of intractable diseases including neurodegenerative conditions. Traditional methods to generate cells for these therapies utilize sequential treatment of signaling molecules to differentiate human pluripotent stem cells into specific cell types. These methods are often inefficient and result in variable cell products. However, one promising recent approach is cell fate reprogramming by expression of single or multiple transcription factors to drive cell differentiation more efficiently. Here we demonstrate our proprietary Cell Squeeze® technology's ability to deliver transcription factors into induced pluripotent stem cells to efficiently generate neurons preclinically. The Cell Squeeze® technology is uniquely positioned to engineer cell function through transcription factor delivery since it enables non-viral, cytosolic delivery of a variety of materials while preserving cell health and avoiding changes to gene expression. Furthermore, the use of the Cell Squeeze® technology allows for the precise timing and magnitude of delivery of one or multiple transcription factors for the delicate task of cell fate reprogramming. This vector-free platform has demonstrated the ability to process billions of cells per minute and is already in use clinically for an oncology target. The efficient non-viral generation of neurons from pluripotent stem cells preclinically illustrates the potential

of using Cell Squeeze® technology to generate specific cell types through transcription factor delivery for cell replacement therapies.

Keywords: Cell replacement therapy, Induced neurons, Microfluidic cell engineering

Poster: 319

LARGE KNOCK IN VIA GENOME EDITING TO ADVANCE PLURIPOTENT STEM CELL APPLICATIONS IN DISEASE MODELING AND CELL THERAPY

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Human induced pluripotent stem cells (hiPSCs) have been globally recognized as a multipurpose research tool for modeling human disease and biology, screening and developing potential therapeutic drugs, and implementing cell and gene therapies. The ability to differentiate human iPSCs into any cell type supports the study of biology and disease in these specified cells in vitro 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. Given the challenges with genome editing efficiency, cell survival and clonal isolation, we have developed a number of reagents and processes to dramatically improve the success rate and timelines for a genome editing experiment in hiPSCs. Key areas of the genome editing workflow that have been addressed include the genome editing tools themselves, the delivery methods and the maintenance of healthy hiPSC cultures during these stressful manipulations. We had previously shown that we had built a reliable approach that reproducibly supports the generation of hiPSC lines carrying small mutations such as SNPs or small deletions. Here, we explored the use of these tools and workflows to insert larger DNA pieces into specific genomic loci to generate fluorescent reporter cell lines for screening or inserting transgenes to mimic disease. Using a small number of loci, we found that introduction of large DNA donors into specific loci was dramatically lower compared to the introduction of SNPs, yet the efficiency obtained was significant enough to allow for the clonal isolation of the edited cells. In summary, we detail advances with tools, reagents and protocols that facilitate the genome editing workflow in hiPSC and demonstrate that the use of such tools can be readily implemented in hiPSC, with potential applications in disease modeling, differentiation optimization and allogeneic cell therapy products.

Keywords: genome editing, pluripotent stem cell, large insertions

12:00 - 13:00 EDT

POSTER SESSION 3

TISSUE STEM CELLS AND REGENERATION

Poster: 330

ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION PLUS FAT GRAFTS FOR HAND THERAPY IN PATIENTS WITH SYSTEMIC SCLEROSIS. RANDOMIZED CONTROLLED CLINICAL TRIAL

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Adipose tissue-derived Stromal Vascular Fraction (ADSVF) has been proposed as regenerative treatment for hand deformities and digital ulcer healing in patients with Sclerosis System. We aimed to evaluate its regenerative properties after local injection of the autologous ADSVF in the hands of these patients. This was an open-label, monocentric, randomized controlled study. Twenty patients diagnosed with SS were enrolled and assigned to the experimental or control group. ADSVF was obtained by enzymatic digestion. ADSVF plus fat micrografts were injected into the right hand of experimental group patients. The control group continued to receive only medical treatment. Digital oximetry, pain, Raynaud phenomenon (RP), digital ulcer healing (DUH), mobility, vascular density of the nail bed, cell surface markers, hand function, and quality of life scores were evaluated in both groups. The mean follow-up period was 168 days. Continuous variables are expressed as median with 95% confidence interval. The differences between before and after the intervention were analyzed with the Wilcoxon range test, and the differences between the control and experimental groups at 0 days and 168 days were analyzed with the Mann–Whitney U test. The range of total viable nucleated cells in the ADSVF was 167.5x106, with viability from 82%. Stem cell markers into the ADSVF was: CD34+ 4.72%, CD45+ 43.9%, CD44+ 36.3%, CD73+ 6.18 %, CD90+ 34.4 %, CD105+ 7.27%, HLA-DR 12.1%, Stromal cells 4.05%. Adverse events were not observed in both groups. There were no changes in disease severity, serologic antibodies, nailfold capillaroscopy patterns, mobility, and hand function in both groups. There were significant improvements in pain, DUH and quality of life scores in the experimental group. RP improved significantly in both groups. However, on statistically comparing the results at 168 days between the groups, significant improvements were only observed in pain levels ($p = 0.02$) and DUH ($p=0.003$). ADSVF administration and micro-grafts were well tolerated. Lowered the pain, the frequency and intensity of the RP, and increased the quality of life in the experimental group. However in the experimental group only the pain and digital ulcer healing improved with statistical significance compared with control group.

Funding Source: Consejo Nacional de Ciencia y Tecnologia (CONACYT) funding this research, with the Sectorial Research Fund in Health and Social Security I0000/739/2017.

Keywords: Adipose derived stromal vascular fraction, Regnerative medicine, adipose mesenchymal stem cells

Poster: 331

LINEAGE TRACING AT SINGLE-CELL RESOLUTION UNVEILS COMPLEX DIFFERENTIATION TRAJECTORIES OF ADIPOCYTE PRECURSORS IN THE SKIN

Rivera Gonzalez, Guillermo, Butka, Emily, Jindal, Kunal, Kong, Wenjun, Waye, Sarah, Hoffmann, Christy, Kamimoto, Kenji, Morris, Samantha

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White adipose tissue (WAT) plays fundamental roles in obesity, type II diabetes, cancer, and normal tissue development. The generation of mature adipocytes, which are the functional cell units that store fat, relies on adipocyte precursors' (APCs) proliferation and differentiation. Therefore, understanding adipocyte precursor differentiation has the potential to identify mechanisms that regulate the formation of fat depots with important clinical implications. However, heterogeneity within the APC population prevents current lineage-tracing tools from tracking and identifying these cells' differentiation process with high resolution. Using single-cell RNA sequencing (scRNA-seq), we revealed the extent of APC heterogeneity in a skin adipogenesis model, identifying two main populations of APCs (progenitors and preadipocytes) that give rise to mature adipocytes in vitro and in vivo. To determine the lineage relationship between progenitors and preadipocytes, we developed a barcode-based cell-tracing assay compatible with scRNA-seq (CellTagging). Using CellTagging, we successfully traced APCs' differentiation paths in their in vivo environment. Furthermore, CellTagging allowed us to identify that progenitor and preadipocyte cells had non-overlapping differentiation trajectories, unveiling a previously unappreciated, complex process in the generation of mature adipocytes. Together, our findings suggest that APCs' identity (progenitor or preadipocyte) determines their differentiation trajectory through particular molecular mechanisms during homeostatic differentiation. Our approach and results can be used to trace APCs' differentiation during non-homeostatic conditions, including models of obesity and white fat beiging.

Keywords: Adipocyte progenitors, Lineage-tracing, single-cell RNA-sequencing

Poster: 332

UNDERSTANDING THE REGULATORY PRINCIPLES OF STEM CELL NICHE ARCHITECTURE IN LIVE MICE

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A regenerative organ's architecture and function require proper stem cell regulation, such as growth factors secreted from the stem cell niche. However, how the niche itself is organized and remodels to support neighboring stem cells' behaviors remains an open area of investigation. Here, we use intravital imaging of cycling hair follicles in live mice to explore how a group of fibroblasts spatiotemporally establishes niche architecture in coordination with epithelial stem cell dynamics. We find that individual niche fibroblasts extend broad membrane protrusions to remodel the entire niche architecture collectively. Surprisingly, despite full fibroblast ablation, the damaged niche still preserves its architecture and remodeling capability, indicating fibroblast survival is not fully necessary for niche architectural maintenance. Furthermore, genetic manipulations show that membrane remodeling RhoGTPase proteins and anchorage to the extracellular matrix through TGFβ signaling are both required for niche fibroblasts to establish proper architectural size and shape. These mutated niche architectures further result in abnormalities in epithelial stem cell activities, hair follicle shape, and hair shaft growth. Altogether, our work reveals essential regulatory mechanisms of niche architecture, providing novel insights into the niche regulation of stem cell activities and organ function.

Funding Source: China Scholarship Council-Yale World Scholars Program

Keywords: hair follicle stem cell niche, tissue architecture remodeling, skin fibroblast dynamics

Poster: 333

DETECTING STEM CELLS FROM HUMAN ADIPOSE TISSUE IN TRANSDIFFERENTIATED TISSUE

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Transdifferentiation mechanism is the transformation of one type of cell to another, which would implicate the involvement of stem cells. 40 samples of renal tissue were treated utilizing GFR Matrix + hEGF + ADSC (stem cells derived from human adipose tissue). We established two study groups; the first one treated with GFR Matrix + AHEGF + ADSC (n = 20) and the control group (n = 20). The transdifferentiated tissue (renal to pancreatic tissue) was positioned intraabdominally in mice for 1 week and after extracted for histological examination. The flow cytometry technique was used, utilizing specific antibodies (CD34, CD105) to establish the existence of stem cells in the transdifferentiated tissue. We noticed cell groups corresponding to CD34-PE, CD105-FITC positive in transdifferentiated tissues placed for a week intraabdominally in mice, and there was no significant difference with the control groups (p = 0.001). The stem cells of the transdifferentiated tissue could have a potential therapeutic application for several illnesses.

Funding Source: Laboratory of Tissue Engineering and Regenerative Medicine, School of Medicine, University of Monterrey, San Pedro Garza García, NL, MEX.

Keywords: Transdifferentiation, Adipose tissue, Pancreatic tissue

Poster: 334

AMNIOTIC MESENCHYMAL STEM CELL SPHEROID-DERIVED SECRETOME BOOSTS REPAIR AND ANGIOGENIC PROCESSES IN HUMAN CARDIAC ENDOTHELIAL CELLS

Derish, Ida[1], Khan, Kashif[2], Zwaig, Jeremy[2], Yu, Bin[3], Cecere, Renzo[4]

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A myocardial infarction (MI) is caused by an obstruction of the coronary arteries of the heart, resulting in necrosis and maladaptive cardiac remodelling. Terminally differentiated cardiomyocytes and cardiac vascular cells are unable to repair the damaged myocardium. Consequences of this pathophysiology often manifest as heart failure in surviving patients, an irreversible cardiovascular disease with no curative therapies. Stem cells are studied due to their cardiac regeneration potential, but trials have yielded insufficient cell engraftment post-injection as well as unreliable differentiation. We examined a novel cell-free strategy utilizing stem cell secretome, which bypasses the limitations of stem cell injection while improving cardiac function. The secretome contains a variety of soluble factors that play a key role in paracrine communication-mediated tissue regeneration and are involved in cell proliferation, angiogenesis and anti-apoptosis. We hypothesized that the more concentrated secretions collected from amniotic stromal mesenchymal stem cell (AMSC) 3-dimensional spheroids (3D) will upregulate repair-promoting biological processes in human cardiac microvascular endothelial cells (HCECs). We found a significant metabolic activity increase in HCECs treated with 3D-derived secretome, compared to HCECs treated with 2D cell culture-derived secretome and untreated controls ($p < 0.0001$). Spheroid-derived secretome caused a higher number of capillary-like HCEC tubules to form ($p < 0.05$). Higher cell migration speed was detected in 3D secretome-treated HCECs ($p < 0.05$) than in 2D secretome-treated HCECs and untreated HCEC controls. Expression of prominent cardiac markers connexin 43, sarcoendoplasmic reticulum Ca2+ ATPase, GATA4 and Troponin T were confirmed in spheroid AMSC cultures via immunocytochemistry, suggesting their cardiogenic potential. Altogether, our data suggests that the AMSC spheroids are a more advantageous source of angiogenesis-boosting secretome than 2D AMSC-derived secretome, with improved metabolic, vasculogenic and cell migration processes in treated HCECs. Our results highlight the potential of this novel and readily harvestable therapeutic agent for cardiovascular repair, to improve patient outcomes and recuperate healthy heart function.

Keywords: Stem Cell Secretome, Cardiovascular Repair, Amniotic Mesenchymal Stem Cell Spheroids

Poster: 335

CUES FROM EXTRACELLULAR MATRIX PROMOTE METABOLIC MATURATION AT EARLY STAGES OF CARDIAC DIFFERENTIATION

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Extracellular matrix (ECM) can directly modulate cell proliferation, migration, and differentiation by mediating diverse growth factors and signaling interactions. Protocols for cardiomyocyte differentiation of induced pluripotent stem cells (iPSCs) that recapitulate cardiac development frequently result in embryonic-like cardiomyocytes, impairing their use in translational heart research. Utilizing the biological capabilities of decellularized ECM (dECM) from porcine myocardium, we established a method to promote an early metabolic maturation of these cells. Using a turbulent agitation device (TAD) we generated small particles (100-500 µm) of porcine ventricular dECM (pvECM). The decellularization process preserved ECM characteristics (expression of laminin, elastin and collagen I confirmed by immunofluorescence) and maintained the architectural morphology (scanning electron microscopy). Matrisome analyses demonstrated that 76.2% of all proteins were core matrisome (proteoglycans, glycoproteins and collagens) related proteins and 23.8% were matrisome-associated proteins (secreted factors, ECM regulators and ECM-affiliated proteins). The addition of pvECM did not impair the cardiac differentiation as we observed ~70% of cTNT+ cells by flow cytometry, consistent with the control group. By immunofluorescence, we observed an intimate interaction of the cardiomyocytes (cTNT) and the pvECM particles (laminin). After 15 days of differentiation, we observed an increase of markers MAPK1, FOXO1, FOXO3, related to cardiac metabolic maturation, and a switch from integrins isoforms ITGA5 and ITGA6, present in immature cardiomyocytes, to integrins isoforms ITGA3 and ITGA7, present in adult cardiomyocytes. We also observed a switch of glucose to fatty acid metabolism in the cardiomyocytes differentiated in the presence of pvECM, with a decrease of glucose uptake and an increase of fatty acid consumption. These findings suggest that dECM powder retained endogenous cues that promoted greater metabolic maturation at earlier stages of cardiac differentiation. Our method using dECM particles during differentiation may be a promising approach to driving more efficient and robust cardiomyocyte maturation.

Keywords: Extracellular matrix, Cardiac differentiation, Maturation

Poster: 336

REPROGRAMMING OF URINE-DERIVED STEM CELLS INTO HUMAN NEPHRON PROGENITOR-LIKE CELLS

Bejoy, Julie[1], Welch, Richard C.[1], Williams, Felisha M.[1], Vanslambrouck, Jessica M.[2], Little, Melissa H.[2], Woodard, Lauren E.[1]

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During embryogenesis, the kidneys develop from intermediate mesoderm including the ureteric bud (UB) and metanephric mesenchyme (MM). The UB progenitors develop into collecting tubules, whereas the progenitors of MM develop to all of the different cell types within the nephron. Even though nephron progenitors have the potential to repair damaged renal tissue, the extraction, and maintenance of human nephron progenitors is ethically and practically difficult. Alternative sources are needed for kidney regenerative medicine strategies. Hendry et al. identified six transcription factors that generate nephron progenitors from human adult cells. We more recently identified a combination of three reprogramming factors (SIX1, EYA1, and SNAI2) sufficient to reprogram adult renal tubule cells to nephron progenitor-like cells. To advance our approach we aimed to use human primary cells that are easier to isolate than tubule cells that require a kidney biopsy. We reprogrammed urine-derived stem cells (USCs) to induced nephron progenitor-like cells (USC-iNPs) through inducible expression from a piggyBac transposon of the three transcription factors SNAI2, EYA1, and SIX1. The reprogrammed cells were cultured in nephron progenitor maintenance media to support the progenitor state of the cells. The resulting USC-iNPs were positive for nephron progenitor markers including SIX2, CITED1, OSR1 by immunofluorescent staining. To evaluate the nephrogenic differentiation potential of USC-iNPs, we used a recombination assay with embryonic mouse kidney. We harvested kidneys from E12.5 Cited1-CreERT2 mice that express eGFP within the Cited1+ cap mesenchyme. Immunostaining of the resulting cell recombination assays revealed that mCherry-positive USC-iNPs localized to proximal tubule-like segments that formed near E-CAD+ distal tubules. We will co-stain the USC-iNPs with additional markers to evaluate the integration of USC-iNPs into the different nephron segments. Further investigation using RNA-seq analysis will be performed to understand the transcriptome of the USC-iNPs. In conclusion, this study supports a method for the generation of nephron progenitors from patient urine samples. The reprogrammed USC-iNPs may provide new mechanisms to understand kidney disease and regenerative therapies.

Keywords: Urine-derived stem cells, Piggybac transposon, Nephron progenitors

Poster: 337

PRELIMINARY CHARACTERIZATION OF CANINE HEPATIC SPHEROIDS: A POTENTIAL MODEL FOR TRANSLATIONAL RESEARCH IN GASTROENTEROLOGY

Gabriel, Vojtech[1], Zdyrski, Christopher H.[2], Dao, Kimberly[3], Sahoo, Dipak K.[4], Iennarella-Servantez, Chelsea K.[4], Bourgois-Mochel, Agnes[4], Jergens, Albert E.[4], Mochel, Jonathan P.[1], Allenspach, Karin[4]

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There are currently no standardized protocols available establishing precise operating procedures for the culture of hepatic spheroids and organoids. Providing such data is key to characterize their normal growth and derive optimal timepoints for passaging, which can prove useful in future experiments focusing on the pathogenesis of select liver diseases. Spheroids were cultured in media as previously described by our group. Hepatic tissue was cultured with or without EDTA incubation before embedding into Matrigel. In total, 845 spheroids derived from one biopsy each of healthy adult beagles were assessed for the first 7 days in culture. The longitudinal (a) and diagonal (b) radius of the spheroids was measured over time to derive the volume (V), surface area (P), 2D ellipse area (A), and circumference (C) of the spheroids as follows: $V \approx 4/3\ \pi ab^2$, $P \approx 2\pi\sqrt{2\ b\sqrt{(a^2+b^2)}}$, $A = \pi ab$ and $C \approx \pi/2\ [a+b+\sqrt{(2(a^2+b^2))}]$. Next, spheroids were collected every day (Day 2-7) for RNA in situ hybridization. Canine probes were designed, and mRNA expression evaluated for the following markers: LGR5 (stem cells), KRT-7 and AQP1 (cholangiocytes), as well as FOXA1 and CYP3A12 (hepatocytes). Liver spheroids grew more consistently if cultured as cell clusters without cellular disruption before culture. Spheroids rapidly expanded in volume, surface area, 2D ellipse area, and circumference as follows: V increased by 479% (± 575%) from Day 2 to 3; P and A increased by 211% (± 208%) and 209% (±198%), respectively, while we noted an increase of 73% (± 57%) in C. Increase in overall volume from day 2-7 was more than 365x, P and A increased 49x and 2D ellipse circumference increased 6x. The optimal time for first passaging of the spheroids was determined to be on Day 7, when most of the spheroids had achieved their maximum volume before transforming into budding organoids. Spheroids expressed preferentially cholangiocyte markers, ranging from 1% to 26% in signal area/total area of cells, and stem cell markers (0.17 to 0.78%), while hepatocyte markers were expressed to a lower extent (0.05 – 0.34% and 0.03 – 0.28% respectively). These preliminary data provide some guidance for the optimal timing of first passaging of hepatic spheroids. Of note, canine hepatic spheroids tend to differentiate into cholangiocytes using our specific media composition.

Funding Source: Iowa State University Brown Graduate Fellowship

Keywords: Spheroid, Canine, Liver

Poster: 338

UNDERSTANDING THE RELATIONSHIP BETWEEN OVARIAN CANCER CELL LINES AND APOBEC3B INHIBITION AS A PATH TO TREATING OVARIAN CANCER

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Ovarian cancer is a lethal gynecologic malignant disease that often goes undetected and is often diagnosed at late stages. It is also known that patients who have been treated for epithelial ovarian cancer (EOC), which is an aggressive type of ovarian cancer, have obtained an enrichment of cancer stem cell population (CSCs) which are accountable for primary tumor growth, metastasis, disease relapse and resistance to chemotherapy. The goal of this research is to focus on the relationship between apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B or A3B), which is an anti-viral DNA deaminase associated with the innate immune system, and ovarian cancer CSCs. A3B is highly expressed in ovarian cancer, especially within EOCs. A3B is a DNA deaminase that causes cytidine to thymidine mutations that lead to drug resistances. Two ovarian cancer cell lines, A2780 and TOV21G, are used to compare the effects between their wild types and their modified versions to better understand A3B function in ovarian cancer CSCs. A2780wt (wild-type), A2780cr (cisplatin resistant), TOV21Gwt (wild-type), and TOV21G A3B KO (knockout) cell lines will be treated with Lynparza (also known as Olaparib), a PARP (poly ADP-ribose polymerase) inhibitor being used in the clinic to treat breast and ovarian cancers. This drug targets DNA repair pathways that occur within cells. PARP serves as a "molecular police" that tracks DNA damaged sequences and mediate repair. Olaparib inhibits one of the two DNA repair pathways that cancer cells lack due to inherited DNA mutations such as BRCA1 and BRCA2. Therefore, the PARP treatment ultimately kills the cancer cells, and the normal cells remain. PARP inhibitor is crucial because it inhibits the pathway that A3B relies on for DNA repair in ovarian cancer. With hopes that this experiment gives us more insight on A3B and ovarian cancer to the treatment of PARP inhibitors, there could be a better understanding if the inhibition of A3B in ovarian cancer cells would be a prominent ovarian cancer treatment path which could eventually be used in organoid and mouse models to further the understanding of A3B inhibition in ovarian cancer.

Funding Source: CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375

Keywords: epithelial ovarian cancer, PARP inhibitor, cancer stem cells

Poster: 339

A DEPARTMENTAL PROGRAM TO SUPPORT AND RETAIN WOMEN FACULTY IN ACADEMIC SCIENCE

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A critical mass of women in leadership positions can bring profound benefits to an organization. In academic science, however, women remain grossly underrepresented at the leadership level. Factors that underpin this discrepancy are wide-ranging and include such issues as i) reduced access to experiences that foster a transition to leadership, ii) the disproportionate burden that women carry that impacts their capacity to invest in their own career growth, iii) organizational cultures that value work above all else, and iii) gender bias, discrimination and harassment. To tackle this complex challenge at the organizational level, we present a holistic program to support and retain women faculty in academic science. The program brings together both men and women in a partnership to explore the underlying factors that limit the professional development of women faculty. This shared partnership is key to the effectiveness of the program. The program also offers practical actions, support and resources to offset these factors. The program is designed to be implemented in a setting where faculty form a cohesive and interdependent group, for example in an academic department, institute or center. It is equally important that this group should be overall committed to addressing gender inequity; however, individual ambivalences and resistance are likely to be encountered. The program can also be adapted to support and retain other groups historically underrepresented in academic science. We present this program as a bona fide example of what a single department, institute or center can do to better support and retain women faculty in academic science, which we hope will serve as a model for others in creating a more diverse and nourishing environment where all memberships are valued.

Keywords: Gender inequity, Policy, Education

Poster: 340

SINGLE-CELL RNA SEQUENCING IDENTIFIES THE HUMAN RETINAL PIGMENT EPITHELIAL STEM CELLS

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The retinal pigment epithelium (RPE) plays a critical role in supporting and maintaining the neural retina. While traditionally considered a homogenous monolayer, differences in morphology, gene expression and function of RPE cells in different retinal regions and the presence of highly proliferative RPE cells indicate RPE cell diversity and the presence of the RPE stem cell (RPESC) subpopulation. To characterize the RPESCs, we isolated RPE cells from adult human eyes and studied the transcriptome of single cells using two single-cell RNA sequencing technologies. We identified 13 RPE clusters, demonstrating significant heterogeneity within the RPE layer. One cluster was identified as the RPESC based on a unique gene expression profile and validated by protein expression in expanding RPESCs in culture. Identifying the RPESC gene expression signature broadens our understanding of retinal function and improves potential for repair to combat RPE dysfunction and vision loss in retinal degenerative diseases.

Funding Source: This study was funded by the National Eye Institute, National Institutes of Health (R01EY029281).

Keywords: Retinal pigment epithelium, RPE stem cells, single-cell RNA sequencing

Poster: 341

PLURIPOTENT STEM CELL-DERIVED CORNEAL ENDOTHELIAL CELLS FOR DONOR-FREE ENDOTHELIAL KERATOPLASTY

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Corneal endothelial dystrophies (CED) are responsible for the majority of corneal transplantations, and although keratoplasty has been effective in treating CED, the global shortage of transplantable-grade donor corneal endothelium (CE) compels for alternative therapies. Here, we evaluate the efficacy of cryopreserved human embryonic stem cell (hESC)-derived corneal endothelial cells (CECs) to form a functional monolayer of CE in mammals (rabbits) and non-human primate (monkeys). We differentiated H9 hESCs to generate CECs under xeno-free conditions. A silicone needle was used to remove the resident CECs in an 8 mm diameter area of the central cornea of rabbits and monkeys. We injected cryopreserved hESC-derived CECs in rabbits and monkeys either immediately after removing the CE or a few days later when corneal edema developed. All clinical models developed transparent corneas 2-3 weeks post CEC-injection and importantly, the thickness and the cell density of the regenerated CE remained comparable to that of the CE in the untreated eye 9- and 12-months post CEC-injection in both rabbits and monkeys. Confocal scanning microscopy confirmed an intact layer of hexagonal/polygonal cells in the CE formed from the cryopreserved hESC-derived CECs. Immunohistochemical (IHC) analysis illustrated intact tight junctions, pump function, and structural integrity of the injected cryopreserved hESC-derived CECs. Finally, all parameters examined during the CBC/blood chemistry analysis were within the normal physiological range while necropsy examination confirmed no remarkable change in multiple tissues examined for teratoma formation. In conclusion, we present evidence of the efficacy of cryopreserved hESC-derived CECs as an alternative to “donor CE” in endothelial keratoplasty.

Funding Source: Maryland Stem Cell Research Fund (SAR)

Keywords: Corneal endothelial dystrophies, Corneal endothelial cells, Endothelial keratoplasty

Poster: 342

DEVELOPMENT OF HIGHLY EFFICIENT MEASLES VIRUS VECTOR FOR REPROGRAMMING OF HUMAN PRIMARY FIBROBLASTS BY TIMELY ELIMINATION OF EXOGENOUS OCT4

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Human fibroblasts can be reprogrammed into pluripotent stem cells (iPSCs) using the four reprogramming factors (RF), OCT4, SOX2, KLF4, and MYC, but the efficiency is low. Of the four RFs, OCT4 is a key mediator of the reprogramming process, but the mechanistic insights into the role of exogenous OCT4 and timelines that initiate the pluripotency network remain to be resolved. Our lab has previously developed a non-integrating RNA virus, Measles Virus (MeV), expressing the four RF in a single vector to produce iPSC. Here, we present the use of MeV vectors for understanding the role of exogenous OCT4 during reprogramming, in the context of mesenchymal-epithelial transition (MET). MET is a hallmark of the reprogramming process in which fibroblasts transition from a mesenchymal to an epithelial phenotype, before gaining pluripotency. We followed the reprogramming of human fibroblasts with MV(O)(SK)(M) vector, in which OCT4 is separated from (SOX2-KLF4) bicistron and analyzed for MET transition. Using qPCR and confocal, we show that the timeline of MET in MeV reprogramming occurred between days 9 and 15, indicating that the timing of MET and reprogramming might be dependent on the type of viral vector used to deliver the RFs. To control the expression of OCT4 during MET, we selected miRNA375 (miR375), a miRNA known to be expressed during MET, and we confirmed its upregulation during the MET phase in the reprogramming of human fibroblasts with MV(O)(SK)(M). We then produced the MV(O)miR375(SK)(M) vector in which we inserted three repeats of the target sequences of miR375 in the 3’ untranslated region of the OCT4 gene, making OCT4 controllable by miR375 expression. The efficiency of reprogramming of MV(O)miR375(SK)(M) is 10 times higher compared to the MV(O)(SK)(M) vector on primary human fibroblasts. Confocal and qPCR analyses of specific genes (EpCAM, CDH-1) and miRNA (miR302a, miR371-miR373 cluster) indicate that the MET occurred 3 days earlier in reprogramming with MV(O)miR375(SK)(M) compared to MV(O)(SK)(M). Hence, for the first time, we show that exogenous OCT4 is only critical for the initiation of reprogramming in the MeV reprogramming and that its timely elimination by MET increases the reprogramming efficiency of primary adult fibroblasts.

Keywords: iPSCs, non-integrating viral vector, Measles Virus Vector

Poster: 343

THE TRANSCRIPTION FACTOR PLAG1 DAMPENS PROTEIN SYNTHESIS IN HUMAN HEMATOPOIETIC STEM CELLS AND PROMOTES THEIR EXPANSION EX VIVO

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Hematopoietic stem cells (HSC) ensure life-long hematopoiesis through the unique functional properties of successive differentiation and sustained self-renewal. Enhancing our understanding of the molecular mechanisms underpinning HSC fate decision-making can inform the development and improvement of HSC-based therapies. To this end, we have identified the zinc-finger transcription factor PLAG1 as a novel enforcer of HSC self-renewal and expansion. Loss of PLAG1

impairs long-term hematopoietic reconstitution by umbilical cord blood-derived (CB) CD34+ hematopoietic stem and progenitor cells (HSPC). In contrast, overexpression of PLAG1 amplifies CB CD34+ HSPC in culture and, as evaluated by limiting dilution xenotransplantation, endows a 15-fold enhancement of functional HSC ex vivo. Consistent with functional outcomes, the PLAG1-enforced transcriptome is positively enriched for signatures of primitive hematopoietic cells. Moreover, pathway analysis of combined RNA-seq and CUT&RUN profiles uncovered negative regulation of ribosome and protein biosynthesis machinery in PLAG1 overexpressing CD34+ HSPC. We functionally demonstrate that PLAG1 overexpression dampens a transient spike in protein synthesis in CD34+ HSPC induced by ex vivo culture, which is concomitant with reduced cell cycle progression and enhanced survival. To further validate the dependency of PLAG1 on translational control we activated translation in HSPC via MYC overexpression and demonstrate attenuation of the effectiveness of PLAG1 to promote in vitro self-renewal. Overall, we have discovered PLAG1 to be among an under-characterized class of protein synthesis regulators in HSPC, and the collection of findings support an emergent paradigm that translational control has unrealized potential for regenerative stem cell applications.

Funding Source: Frederick Banting and Charles Best Canada Graduate Scholarships

Keywords: Hematopoietic Stem Cell Expansion, Translational Control, Xenotransplantation

Poster: 344

GCSF INDUCED MOBILIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM THE EMBRYONIC HEMATOPOIETIC NICHE

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Hematopoietic stem cell transplantation requires a collection of hematopoietic cells from patients or stem cell donors. GCSF is widely used in the clinic to mobilize hematopoietic stem and progenitor cells (HSPCs) from the adult bone marrow niche into circulation, allowing a collection of HSPCs from blood. Many interactions between hematopoietic stem cells and adult niche are also found in the embryonic hematopoietic niche. To study if GCSF can mobilize HSPCs from the embryonic hematopoietic niche, we expressed GCSF under the control of heat-inducible promoter in zebrafish embryos. GCSF was induced with heat-shock at three days post-fertilization when HSPCs reside in the caudal hematopoietic tissue (CHT), the embryonic hematopoietic niche of zebrafish. Live imaging Tg(runx:mCherry) reporter line of HSPCs 24 hours post GCSF induction demonstrated that circulating HSPCs increase in response to GCSF expression. Whole-mount in situ hybridization of c-myb displayed HSPCs that prematurely colonize the kidney, the adult hematopoietic niche of zebrafish, in 73% (n = 41/56) of embryos when GCSF is expressed. Together, these data suggest that HSPCs leave the embryonic hematopoietic niche in response to GCSF, enter circulation, and colonize the adult hematopoietic niche. Expansion and activation of neutrophil is one of the mechanisms proposed for how GCSF mobilizes HSPCs from the bone marrow. To verify whether neutrophil is necessary for GCSF induced mobilization from the CHT, we ablated neutrophils with a nitroreductase based system in embryos. Regardless of the neutrophil ablation, we observed an increase in circulating HSPCs and premature colonization of the kidney 24 hours post GCSF expression in 77% (n = 36/47) of embryos. A number of EdU positive proliferating HSPCs did not change significantly in response to GCSF, demonstrating that HSPC proliferation is not affected by GCSF. These results demonstrate that GCSF is sufficient to mobilize HSPCs from the embryonic hematopoietic niche in a neutrophil-independent manner.

Funding Source: NIH 1RC2DK120535-01A1

Keywords: Mobilization, Hematopoietic stem and progenitor cell, Embryonic hematopoietic niche

Poster: 345

KINETIC STEM CELL (KSC) COUNTING ALGORITHMS FOR DETERMINATION OF TISSUE STEM CELL SPECIFIC NUMBER AND DOSE

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Since the earliest beginnings of tissue stem cell biology, the field has lacked a convenient method for routine, accurate, and precise determination of the specific fraction of homeostatic renewing stem cells found in isolated perinatal or postnatal tissue cell preparations. Only SCID mouse repopulating cell (SRC) assays have had sufficient cell-type specificity to estimate the stem cell-specific fraction. However, SRC assays are only applicable for hematopoietic stem cells; and they have many shortcomings that limit their development for routine use to determine stem cell number for research and stem cell dose for medicine. Recently, we reported computational simulation software (PSCK-RIFS) with the ability to determine the specific stem cell fraction (SCF) in diverse vertebrate tissue cell preparations, including human. The underlying biological basis for the software is "kinetic stem cell counting." The PSCK-RIFS software defines the number of tissue stem cells responsible for the unique cumulative population doubling (CPD) kinetics of long-term serial cultures of evaluated tissue cell preparations. Using the software, we discovered mathematical algorithms that allow calculation of the SCF of primary tissue cell preparations by inputting routine population doubling time data. We now report the discovery or related algorithms that define the half-life for the decay of SCF with increasing CPDs of tissue cell preparations. The newly defined SCF half-lives constitute state functions tissue stem cells. They are quantitatively distinctive for stem cells of different tissue origin and define the SCF of a tissue cell preparation at any future time in its cell culture history.

Funding Source: National Heart, Lung, And Blood Institute of the National Institutes of Health under Award Number R43HL154900 BioFabUSA Technology Project Award granted by the Advanced Regenerative Manufacturing Institute, Inc.

Keywords: tissue stem cell, stem cell count, stem cell dose

Poster: 346

PRECLINICAL DEVELOPMENT OF ROR1-TARGETED CAR T-CELL THERAPY FOR MULTIPLE MYELOMA

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Multiple myeloma is a fatal plasma cell neoplasm characterized by malignant expansion of abnormal antibody-producing cells. Despite recent therapeutic advances, most patients relapse due to uncontrolled regeneration of malignant progenitor-like cells in protective, inflammatory microenvironments. However, no clinical therapies currently target cancer stem cells (CSCs) in myeloma. The fetal oncogene ROR1 (receptor tyrosine-kinase like orphan receptor-1), a pseudokinase that activates non-canonical Wnt signaling and the NF-kB pathway during normal human embryonic development, is associated with CSC expansion in B-cell leukemias and lymphomas. Accordingly, ROR1 is a promising therapeutic target because it may promote the malignant growth and self-renewal of myeloma progenitor populations. Our gene expression analyses and clinically-relevant functional flow cytometry assays confirm ROR1 mRNA and receptor protein expression in several human myeloma cell lines and in primary multiple myeloma patient samples. ROR1 expression is further enriched in vitro with acquired resistance to clinical anti-myeloma drugs. In addition, overexpression of interferon regulatory factor-4 (IRF4), a myeloma cell-enriched transcription factor that we recently showed promotes myeloma regeneration, increased human ROR1 expression levels. We have recently created a series of anti-ROR1 CAR T-cells that employ an scFv generated from the fully humanized ROR1-specific monoclonal antibody cirmtuzumab. Preclinical assays were performed using Incucyte fluorescent real-time cell analysis of human myeloma RPMI-8226 cells stably expressing luciferase and RFP. These cells expressed endogenous ROR1 and were sensitive to in vitro cell-mediated killing by anti-ROR1 CAR T-cell treatment at effector to target (E:T) cell ratios as low as 0.33:1. In feasibility studies facilitating the assessment of anti-ROR1 CAR T-cell activity in vivo, we developed unique cell line xenograft and patient-derived xenograft models that faithfully recapitulate features of high-risk myeloma and engraft abundant human malignant plasma cells expressing endogenous ROR1. Altogether, these results set the stage for the development of clinically-relevant ROR1-targeted therapeutic and diagnostic modalities for multiple myeloma patients.

Keywords: multiple myeloma, cancer stem cells, ror1

Poster: 347

ASPARTATE IS LIMITING FOR MOUSE HEMATOPOIETIC STEM CELL FUNCTION

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A key function of the electron transport chain is to promote aspartate synthesis, which is required for cancer cell proliferation. However, it is unclear whether aspartate is also limiting in normal stem cells, which divide intermittently. We found that hematopoietic stem cells (HSCs) do not take up exogenous aspartate. To test if aspartate limits HSC function, we over-expressed the glutamate/aspartate transporter, Glast, or deleted glutamic-oxaloacetic transaminase 1 (Got1). Each increased aspartate levels in hematopoietic stem/progenitor cells, increasing the function of HSCs but not colony-forming progenitors. Conversely, deletion of glutamic-oxaloacetic transaminase 2 (Got2) reduced aspartate levels and HSC function but not colony-forming progenitors. Isotope tracing showed aspartate was used to synthesize asparagine and purines. Both contributed to increased HSC function as deletion of asparagine synthetase (Asns) or treatment with 6-mercaptopurine attenuated the increased function of GLAST over-expressing HSCs. Stem cell function is thus limited by aspartate in some contexts.

Keywords: aspartate, asparagine, purine

Poster: 348

NOTCH/IL21 AXIS PROMOTES PRE-THYMIC T CELL PROGENITOR DEVELOPMENT AND EXPANSION

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A major clinical obstacle after treatment of blood disorders with hematopoietic stem cell transplantation (HSCT) is the resultant long-term impairment in T cell mediated adaptive immunity. Though T cell development in the thymus has been extensively characterized, there are significant gaps in our understanding of pre-thymic T cell potential. We have uncovered a Notch/IL21 signaling axis which is involved in the priming of bone marrow Common Lymphoid Progenitor (CLP) cells towards the T cell fate. Using a transgenetic Notch1 hypomorhic mouse model and unbiased differential expression analysis (RNAseq), we detected a severe decrease in the expression of the IL21-receptor (IL21r) in the Notch hypomorph cells compared to WT CLPs. In vivo treatment with IL21 induced a rapid expansion of Notch-responsive CLPs. Analysis of BM populations showed that tissue resident memory CD4+ T cells are responsible for BM IL21 production. Taking advantage of this novel signaling axis, we have generated T cell progenitors ex vivo which better repopulate the thymus and peripheral lymphoid organs of irradiated recipient mice. Importantly, Notch and IL21 activation is equally effective in the priming and expansion of human cord blood cells toward a T cell fate, confirming the translational potential of the combined treatment.

Keywords: Common Lymphoid Progenitor, T cell development, Notch signaling

Poster: 349

GENERATION OF OFF-THE-SHELF UNIVERSAL HEMATOPOIETIC CELLS FOR ALLOGENEIC CELL THERAPIES

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Cell-based therapies utilizing hematopoietic cells - given their vast range of functions - have the potential to treat numerous devastating diseases. However, as with all cell-based therapies, the fundamental challenges of safety and allogenic acceptance are crucial hurdles that need to be overcome. We have previously integrated the eight immunomodulatory transgenes of the iACT system into human SafeCell (SC) ESCs to prevent allograft rejection without the use of any immunosuppression. The SC system combined with iACT (SC-iACT cells) has proven to be compatible with generating a spectrum of potential therapeutic cells. However, the overexpression of immunomodulatory iACT genes are expected to interfere with the differentiation to certain hematopoietic lineages. Here we show that differentiated cells derived from SC and SC-iACT ESCs express myeloid markers. However, only SC differentiated cells are capable of phagocytosing heat treated E.coli. This suggests that cells expressing iACT transgenes are not able to differentiate into functional alveolar-like macrophages in vitro. To overcome this roadblock, we are aiming to temporarily block some of the iACT transgenes during the differentiation process using small molecule inhibitors or genetic approaches. Ultimately, the generation of functional hematopoietic SC-iACT cells will advance the development of future cell-based therapies while overcoming the challenges of safety and allogenic acceptance.

Funding Source: Medicine by Design

Keywords: Hematopoietic, cell-based-therapies, allogeneic

Poster: 350

TISSUE CHIPS IN SPACE: USING MICROGRAVITY TO STUDY IMMUNOLOGICAL SENESCENCE AND ITS IMPACT ON TISSUE REPAIR BY MESENCHYMAL STROMAL CELLS

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Aging is associated with dysregulation of the immune response, which is also termed “immunosenescence.” Although each part of the immune system is altered to some extent by the aging process, the adaptive immunity and especially T cells seem more extensively affected. In fact, the number and proportion of late-differentiated T cells (so called TEMRA cells), is higher in the elderly than in the young and their accumulation may contribute to the enhanced systemic pro-inflammatory milieu commonly seen in elderly individuals, which impairs wound and bone fracture healing. Using an earth-bound system for simulated microgravity (µg), we showed that T cells from healthy volunteers (n=3) markedly increase their proportion of TEMRA cells. This effect occurred within 72h and resulted in significant upregulation of TEMRA markers such as CD57 and loss of CD27, CD28. We had therefore identified a model for accelerated T cell senescence with implications for space travel. Since T cells are in contact with all body niches including tissue- and organ-specific stem cells, we next investigated how µg-induced T cell senescence impacts the physiology of mesenchymal stromal cells (MSCs). We used iPSC technology to differentiate MSCs from the same healthy T cell donors and studied changes in MSC characteristics in co-culture. Under simulated µg conditions, MSCs in co-culture showed impaired repair capacity in wound healing assays when compared to 1g controls. This impact in co-culture was significantly stronger than the direct effect of simulated µg on MSCs alone, supporting the notion that TEMRA cells impair MSC function. To validate our results in real µg, we sent 46 tissue chips aboard SpaceX16 to the International Space Station. They arrived on December 5th 2018 and returned frozen to Earth on January 11th 2019. RNA sequencing of MSC-T cell co-cultures showed variances primarily driven by the presence of T cells in culture and on the µg condition. While µg might be a favorable environment for expanding MSCs in vitro, their intrinsic wound healing capacity seems impaired when co-cultured with T cells on tissue chips. This finding has implications for injuries or surgeries in space and sheds light on the broader effects of T cell senescence on tissue repair.

Funding Source: NIH/CASIS

Keywords: Tissue Stem Cells, Aging, Immune system

Poster: 351

MESENCHYMAL PROGENITOR CELLS IN MURINE DIGIT TIP AMPUTATION REPAIR ARE MORE SIMILAR TO ABERRANT WOUND HEALING THAN BLASTEMA MEDIATED LIMB REGENERATION AND DEVELOPMENT

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Distal digit tip amputation (DTA) repair is proposed as mammalian regeneration via blastema. However, studies have not compared cellular programs after DTA to development, blastema-mediated regeneration, or aberrant wound repair such as traumatic heterotopic ossification (HO), in which ectopic bone forms post injury. Both DTA repair and HO result in de novo bone and arise from Pdgfra+ mesenchymal progenitor cells (MPCs). True regeneration via blastema occurs in axolotls, which regenerate amputated limbs. We hypothesized that transcriptional profiles and trajectories of DTA MPCs are more like HO and less like embryonic digit development (EDD) and axolotl limb regeneration. Using lineage tracing and single cell RNA sequencing transcriptomics, we analyzed MPC fate differentiation in EDD (E11, E14, P3, 7961 cells; GSE135985), DTA repair (0, 10 & 14 days post DTA, 3126 cells, GSE135985), and HO (0, 7 & 21 days post injury, 8700 cells, GSE126060) with Seurat 3.1.1 and identified the most upregulated genes in MPCs

vs. non-MPCs. These were compared to known upregulated genes in the axolotl blastema. In the axolotl, upregulated blastema genes include the mouse orthologs kazald1, cirbp, and rbm3, which were not upregulated in MPCs after DTA or HO. The regeneration organizing cell (Lef1+/Tp63+ [mouse ortholog: Trp63]), required for Xenopus tadpole tail regeneration, was also not present in DTA repair or HO. After injury, HO and DTA MPCs shared 4/6 top upregulated genes (Col1a1, Dcn, Col1a2, Lum, p<10-6). When cells post injury in DTA and HO were combined and clustered, MPCs had similar UMAP dimensions, suggesting similar transcriptional profiles. Trajectory analysis (Monocle 2) showed MPCs in DTA and HO localized to similar regions on the trajectory after injury. Cells converged on osteogenic (Alpl+) and chondrogenic (Acan+) branches. Conversely, HO and EDD MPCs had little overlap and distinct trajectories. DTA and EDD MPCs are also known to be distinct. Together, this data indicates MPCs in DTA repair and HO are transcriptionally distinct from MPCs in true blastema-mediated limb regeneration and development. MPCs in DTA repair and HO are transcriptionally similar and acquire similar trajectories post injury. This suggests MPC programming responsible for DTA repair is more like HO than regeneration via blastema.

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Keywords: mesenchymal progenitor cell, blastema, digit tip amputation

Poster: 352

LOSS OF VITAMIN A METABOLISM AND RETINOIC ACID SIGNALING IN AGING DRIVES MURINE MUSCLE STEM CELL DYSFUNCTION

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In aging, muscle stem cells (MuSCs) dwindle in number and function, leading to impaired tissue healing and muscle atrophy. Several intrinsic mechanisms have been identified that contribute to MuSC dysfunction in aging, such as loss of proteostasis, aberrant chromatin packaging, and altered cellular metabolism. However, the extrinsic signaling factors that confer these modifications have been underexplored. We have performed integrative genomic analysis of young and aged MuSCs and observed genes related to Vitamin A (VA) metabolism and retinoic acid (RA) signaling, the active metabolite of VA, are downregulated in aged MuSCs. The role of the VA pathway in MuSC aging has not been evaluated, nor has the role of RA signaling in supporting MuSC contributions to muscle regeneration and muscle innervation across lifespan. We show, through the dietary depletion of VA in young mice, that VA is necessary in maintaining quiescence, restraining activation, protecting against oxidative damage due to reactive oxygen species (ROS), and modulating the nuclear envelope and chromatin organization in MuSCs. We also examine the effects of VA depletion in the ability of MuSCs to engraft at the neuromuscular junction and differentiate into post-synaptic myonuclei to support reinnervation following sciatic nerve injury. Finally, we show that the pharmacological repletion of RA signaling helps ameliorate these MuSC deficiencies in aged mice. These findings–collected using a variety of in vitro and in vivo assays, as well as robust bioinformatics analyses–provide critical new insights into the mechanisms that drive muscle aging and will help inform clinical therapies intended to combat muscle wasting in aging populations.

Keywords: muscle stem cells, aging, neuromuscular junction

Poster: 353

MUSCLE STEM CELL HOMESTASIS REGULATION BY SESTRINS

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Skeletal muscle is preserved by a population of stem cells called satellite cells, but in aging these cells exhibit reduced capacity to function. As such, understanding genes and pathways that protect satellite cells during aging are needed. Sestrins are a class of stress-inducible proteins that act as antioxidants and inhibit the activation of the mammalian target of rapamycin complex 1 (mTORC1) signaling complex. However, the role of Sestrins has not been explored in adult stem cells. Herein, we show that Sestrin1,2 loss in young satellite cells results in hyperactivation of the mTORC1 complex, increased propensity to enter the cell cycle, and oxidative stress metabolism. These changes suggested Sestrins preserve satellite cell homeostasis and to further evaluate this effect, Sestrin1,2 knockout mice were aged to 14 months. Aged Sestrin1,2 knockout mice demonstrated a loss of satellite cells and reduced ability to regenerate when challenged by repetitive injuries. Integrating our data suggest Sestrins are important regulators of satellite cell metabolism and may be strong targets for protecting stem cells from aging.

Keywords: Sestrins, Satellite Cells, skeletal muscle

Poster: 354

IN VITRO EXPANDED SKELETAL MYOGENIC PROGENITORS FROM PLURIPOTENT STEM CELL-DERIVED TERATOMAS HAVE HIGH ENGRAFTMENT CAPACITY

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One major challenge in realizing cell-based therapy for treating muscle-wasting disorders is the difficulty in obtaining therapeutically meaningful amounts of engraftable cells. We have previously described a method to generate skeletal myogenic progenitors with exceptional engraftability from pluripotent stem cells via teratoma formation. Here, we showed that these cells are functionally expandable in vitro while retaining their in vivo regenerative potential. Within 37 days in cultures, teratoma-derived skeletal myogenic progenitors were expandable to a billion fold. Similar to their freshly sorted counterparts, the expanded cells expressed PAX7 and were capable of forming multinucleated myotubes in vitro. Importantly, these cells remained highly regenerative in vivo. Upon transplantation, the expanded cells formed new DYSTROPHIN+ fibers that reconstitute up to 40% of tibialis anterior muscle volume and

repopulated the muscle stem cell pool. Our study thereby demonstrates the possibility of producing large quantities of engraftable skeletal myogenic cells for transplantation.

Keywords: Pluripotent stem cells, Myogenic differentiation, Muscle stem cells

Poster: 355

GASTRIC PACEMAKER STEM CELL AGING

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Gastric dysfunction in the elderly may cause reduced food intake, frailty, and increased mortality. Interstitial cells of Cajal (ICC) serve as pacemaker and neuromodulator cells in the gastrointestinal tract. ICC decline with age in humans, and loss of ICC contributes to gastric dysfunction in progeric klotho mice hypomorphic for the anti-aging Klotho protein which attenuates Wnt (wingless-type MMTV integration site) signaling. The molecular mechanisms of age-related ICC depletion remain unclear. We tested the hypothesis that Wnt-induced up-regulation of transformation related protein (TRP53) in ICC stem cells (ICC-SC) contributes to age-related ICC loss. Mice aged 1-107 weeks, klotho mice and cell lines of mouse ICC-SC were studied by RNA sequencing, immunoblots, histochemistry, flow cytometry, ethynyl/bromodeoxyuridine incorporation, and ex-vivo gastric compliance assays. The klotho and aged (18-24 months old) mice showed similar ICC loss and impaired gastric compliance, indicating impaired ability of stomach to relax in response to filling. ICC-SC decline preceded ICC depletion. Canonical Wnt signaling and TRP53 increased in gastric muscles of klotho and aged mice. Overstimulated canonical Wnt signaling increased DNA damage response and TRP53 protein and reduced ICC-SC self-renewal. TRP53 induction by nutlin 3a persistently inhibited G1/S and G2/M cell cycle phase transitions without activating apoptosis, autophagy, cellular quiescence, or canonical markers/mediators of senescence. G1/S block reflected increased cyclin-dependent kinase inhibitor 1B and reduced cyclin D1 from reduced extracellular signal-regulated kinase activity. In conclusion, increased Wnt signaling causes age-related ICC loss by up-regulating TRP53, which induces persistent ICC-SC cell cycle arrest without up-regulating canonical senescence markers. These results suggest that stimulation of ERK-mediated signaling pathways could treat age-related ICC-SC/ICC depletion and gastric dysfunction.

Funding Source: National Institutes of Health grants R01 DK058185, R01 DK121766, P01 DK068055, P30 DK084567, F31 DK089974, AGA–Allergan Foundation Pilot Research Award, and the Mayo Clinic Center for Individualized Medicine.

Keywords: aging, gastric pacemaker, cell cycle arrest

Poster: 356

REGENERATIVE NEURAL STEM CELLS ARE INHIBITED BY TGFBETA1 INDUCED EXPRESSION OF GPNMB ON DEMYELINATION

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Endogenous repair pathways of the central nervous system are currently insufficient to repair the CNS following injury or degeneration. Specifically, pools of multipotent neural stem cells (NSCs) capable of generating new neurons, astrocytes, and oligodendrocytes exist within the central nervous system (CNS), however these and other cell types often fail to fully repair CNS injuries or degeneration. During neurodegenerative diseases such as multiple sclerosis, cytokines such as TGFbeta1 (TGFb1) increase in the CNS, however the effect of this expression on NSC mediated repair is not fully understood. We have identified a novel target of TGFb1 in NSCs called glycoprotein non-metastatic melanoma b (Gpnmb), which negatively regulates that recruitment and differentiation of the sonic hedgehog responsive Gli1+ NSCs. TGFb1 directly stimulated Gpnmb expression in NSCs in vitro, via activation of the downstream TGFb1 transcription factor complex Smad3/4 and binding to the putative Gpnmb promoter. Expression of Gpnmb in NSCs in vitro resulted in decreased oligodendrocyte generation, while inhibiting Gpnmb reversed this effect. To test the effect of Gpnmb modulation in vivo, Gpnmb-LacZ mice were generated and bred to homozygosity to created functional Gpnmb-Null mice. These mice develop normally, and following cuprizone mediated demyelination there was an increase in oligodendrocyte lineage cells. To confirm this increase is results from increased recruitment of Gli1+ NSCs and is mediated by TGFb1 signaling, we obtained TGFbeta-Receptor subunit 2 (TGFb-R2) floxed mice, to knockout TGFb1 signaling in neural stem cells. These mice were bred to Gli1-CreERT2;Ai9 reporter mice to fate-map Gli1+ NSCs while eliminating TGFb-R2 expression. Following chemical mediated demyelination, mice lacking TGFbeta-R2 in Gli1+ NSCs showed enhanced recruitment of NSCs following demyelination, and increased oligodendrocyte generation. Our data identifies a novel regulatory pathway of adult NSCs, where demyelination derived TGFb1 activates Gpnmb in NSCs and inhibits the recruitment and generation of new oligodendrocyte lineage cells.

Keywords: Regeneration, neural stem cell, oligodendrocyte

Poster: 357

LOW-DOSE METHYLMERCURY PROMOTES PRENATAL DIFFERENTIATION OF MOUSE CORTICAL PRECURSORS BY STIMULATING CREB ACTIVATION

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Methylmercury (MeHg) is a dangerous pollutant that has been shown to elicit a range of adverse health effects in both humans and wildlife populations. Additionally, MeHg is able to cross the blood-brain-barrier and placenta and cause

neurodevelopmental defects in the developing fetus. Research from our lab has demonstrated that nanomolar exposure to MeHg can alter embryonic cortical precursor (CP) development. We showed that cultured embryonic CPs exposed to 250nM MeHg increased neuronal differentiation at the expense of CP population. The safe limit of MeHg permitted within commercially available fish in Canada is currently 0.5 ppm. Our research aims to determine underlying cellular and molecular mechanisms that causes MeHg-induced premature differentiation. Here we showed that 250nM MeHg promotes direct neuronal differentiation from CPs by stimulating CREB activity, measured through upregulated phospho-serine 133 (p-S133) CREB. This elevated premature differentiation can be alleviated through repulsive interaction between CREB and CREB Binding Protein (CBP). We further performed single-cell RNA-seq using E13.5 cortices isolated from embryos whose mother received either 0.2ppm MeHg or mock control through drinking water. Our single-cell RNA-seq data analysis showed that 0.2ppm MeHg enhanced asymmetrical division of proliferative radial glial cells and upregulated downstream CREB target genes in proliferative radial glial cells, potentially leading to increased neuronal differentiation during development. These novel findings demonstrate that exposure to low-dose MeHg poses a novel threat to fetal brain development and that there may be a potential therapeutic strategy to alleviating heightened CP differentiation.

Keywords: Cortical Development, Prenatal Exposure, Toxicology

Poster: 358

DYNAMICS OF CELL CYCLE PROTEINS IN STEM CELL HOMEOSTASIS AND NEURAL DEVELOPMENT

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Neural development requires the coordinated replication of cells and histogenic pathways that eventually leads to the formation of complex cell and tissue types. While this requires the coordinated expression of proteins that control DNA replication and cell commitment, the processes that govern each process is not well understood. Given that tissue formation requires the coordinated proliferation and differentiation of cells a greater understanding of the proteins that control cell division at different stages of development represents an important first step in understanding neural development. To address this, we have developed a human stem cell based Fluorescent Ubiquitination based Cell Cycle Indicator (FUCCI) to visualize the development, homeostasis, and differentiation of stem cells and developing neural tissues. To generate these FUCCI iPSC lines we used the CRISPR/Cas9 gene-editing to integrate a constitutively active FUCCI expression cassette into the CLYBL safe harbor site. This system uses phase specific expression of red (mRuby3) or green (EGFP) fluorescent proteins that are present in G1 and S,G2,M stages of the cell cycle respectively. Since these proteins are fused with Cdt1(30-120) and geminin (1-110) domains there are targeted for degradation by ubiquitin specific ligases in opposite phases of the cell cycle. We next used fluorescence-activated single cell sorting (FACS) to isolate FUCCI cells at different stages of the cell cycle in order to characterize and compare gene expression profiles in stem cells and neural progenitors. Through this process, we will gain a better understanding about the gene expression patterns that lead to early stages of neural tissue formation.

Funding Source: Shiley Eye Institute, University of California San Diego, CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375

Keywords: human pluripotent stem cells, CRISPR/Cas9, FUCCI

Poster: 359

EARLY LOW-DOSE EXPOSURE OF METHYLMERCURY PROMOTES PREMATURE NEURONAL DIFFERENTIATION IN MOUSE EMBRYO AND IMPAIRS POSTNATAL CORTICAL DEVELOPMENT

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Methylmercury (MeHg) is an environmental neurotoxicant affecting health of millions of people globally. Many studies showed that early-life exposure of MeHg can lead to neurological deficits in the offspring later in the life. However, it remains unknown about its underlying mechanisms contributing to these neurological deficits. Here, we asked whether the low-dose MeHg exposure to pregnant dam in vivo has impacts on embryonic and postnatal cortical development. We first treated pregnant mice with 0.02 ppm or 0.2 ppm MeHg via drinking water starting from the first day of their pregnancy until embryonic day 15 (E15). We found that there was a significant decrease in the number of cortical precursors (PAX6+) and a robust increase in the number of neuroblasts (DCX+) in the ventricular zone (VZ) of the 0.2ppm MeHg-treated embryos. However, the numbers of intermediate progenitors (TBR2+) and cortical neurons (TBR1+) were not altered by 0.2ppm MeHg treatment. We further investigated how prenatal MeHg exposure affects postnatal neurogenesis. When pregnant mice were treated with 0.2 ppm MeHg until postnatal 7 (P7), 0.2 ppm-treated pups showed significant increase in the DCX+ neuroblasts (DCX+) in the subventricular zone (SVZ). In addition, 0.2 ppm MeHg also caused robust increase in TBR1+ neurons in cortical layer 5. Our results suggest that prenatal low-dose exposure of MeHg disrupts embryonic neurodevelopment by the promotion of premature neuronal differentiation and depletion neural stem cell pool in embryo, leading to impairment in postnatal cortical development. Metformin is an FDA-approved drug. Recently our lab found that it could revere the impaired neuronal differentiation of cortical precursors caused by MeHg in vitro. Our current work is focusing on metformin's protective effects in vivo. Overall, our study provides insight into the potential mechanisms for the early mercury exposure during fetal development having impact on postnatal brain development.

Keywords: Neurodevelopment, Neurogenesis, Methylmercury

Poster: 360

PREGNANCY RESEMBLES HETEROCHRONIC PARABIOSIS AND AMNIOTIC FLUID EXTRACELLULAR VESICLES TRANSFER REGENERATIVE POTENTIAL

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Aging is an evolutionary conserved mechanism driving genetic diversity in all eukaryotic organisms. This process of "timing out" individuals allows resources to be made available to "novel" genetic variants (offspring) potentially better suited to take advantage of local environments or to inhabit new. Aging progresses due to a gradual, but continuous, depletion of endogenous stem cells, which are capable of maintaining homeostasis through tissue repair. As a consequence, aged individuals experience progressively slower and less complete tissue repair. This process has been interrupted in murine laboratory experiments referred to as heterochronic parabiosis, where an old individual regains youthful repair potential when connected to a young individual's blood supply though a common dermal patch. This phenomenon is thought to happen by boosting the regenerative capacity of endogenous tissue stem cells. A natural counterpart exists and that is pregnancy. Pregnancy is an unusual form of heterochronic parabiosis, as the placenta prevents most blood cells to be exchanged between the young and the older parabionts. Instead, plasma, including small extracellular vesicles (EVs), can readily cross the placental barrier. These nanosized EVs, which accumulate in the amniotic fluid, are essential for fetal organogenesis and growth and also impact the mother, as they are essential for maternal physiological changes in response to the stresses of pregnancy. Using an array-based multiplex ELISA approach we have identified over 200 bioactive cytokines, proteases and soluble receptors related to inflammation and tissue repair from purified EVs derived from human amniotic fluid (hAFEVs). We tested the hypothesis that these EVs are capable of improving age-related pathology by employing a murine model of chronic inflammatory mono-arthritis of the knee. Through intra-articular injection of hAFEVs we learned that hAFEVs were immunologically tolerated and reduced knee-inflammation and promoted tissue repair in treated animals (n=12) compared to control animals (n=8) up to 21 days post injury. These data suggest a potential strategy for accessing benefits of heterochronic parabiosis to boost the regenerative capacity of endogenous tissue stem cells in humans and regain the homeostatic repair potential of the young.

Keywords: Aging, heterochronic parabiosis, amniotic fluid

00:00 - 1:00 EDT

POSTER SESSION 4

NEW TECHNOLOGIES

Poster: 401

INDUCTION OF NEURAL CREST LINEAGE CELLS FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS WITH SMALL MOLECULAE COCKTAILS

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Neural crest cells (NCCs) are expected to be a promising source for cell therapy and regenerative medicine for its capability of multipotency, self-renewal capacity, and releasing various trophic factors. While NCCs are broadly distributed over the whole body, induction of the cells and its lineage are difficult. In this study, we attempt to directly induce neural crest cells with small molecule cocktails using human adipose-derived mesenchymal stem cells (ADMSCs). We identified that two step application of 6 small molecules for 6 days could induce human ADMSCs into nerve growth factor receptor (NGFR)- and sex-determining region Y-box 10 (SOX10)-positive NCCs by immunofluorescent and qPCR analysis. In addition, mRNA expressions of various trophic factors were significantly upregulated in induced cells following small molecular treatment. Cell surface maker profiling also suggested dedifferentiation from ADMSCs and induction into NCCs. These results indicate that our small molecular treatment method can induce neural crest lineage cells directly dedifferentiation of human ADMSCs into NCCs, and thus would offer a promising experimental platform for studying human NCCs for application to cell therapy and regenerative medicine.

Funding Source: Funding was provided from Japan Society for the Promotion of Science (JSPS) to YT and YSK. Funding was also provided by BioMimetics Sympathies Inc. to YSK.

Keywords: mesenchymal stem cells, neural crest cells, small molecular cocktails

Poster: 402

AN EVOLVED AND ENHANCED POU REPROGRAMMING FACTOR FOR CELL FATE ENGINEERING

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Cellular reprogramming has the potential to revolutionize medicine through personalized disease models and regenerative therapies. But before it reaches clinical application, cell fate conversions need to be efficient, fast, scalable, and reproducible. Engineering the scaffold of the Pit-Oct-Unc (POU) factor in the context of iPSC reprogramming, we identified an enhanced POU factor (ePOU) that substantially outperform wild-type Oct4 in terms of speed, efficiency, synergizes with re-engineered Sox factors. ePOU can induce pluripotency with Sox2 alone and in the absence of Sox2 in three factor - ePOU/Klf4/c-Myc cocktails. To elucidate the mechanism behind its enhanced activity, we performed biochemical and biophysical assays as well as studied genome-wide binding, regulation, nucleosome binding and chromatin dynamics. We found that ePOU acquires a new preference to dimerize on palindromic DNA elements. Compared to Oct4, ePOU is thermodynamically stabilized. ePOU reveals an unappreciated role of thyrotropin-releasing hormone signaling and binds a distinct class of retrotransposons. Collectively, these features enabled ePOU to accelerate the establishment of the pluripotency network. We anticipate that this work will facilitate the engineering of factors in more challenging reprogramming systems in the future.

Funding Source: National Natural Science Foundation of China (Grant No. 31771454) Research Grants Council of Hong Kong General Research Fund (RGC/GRF) projects number 17128918 and 17101120, G-HKU701/18 Health and Medical Research Fund (06174006)

Keywords: Reprogramming, Protein engineering, POU

Poster: 403

EVALUATING GRNA SCAFFOLD DESIGN FOR SINGLE-CELL CAPTURE CRISPR-ACTIVATION HESC DIFFERENTIATION

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The combination of CRISPR perturbations with single-cell RNA sequencing readout represents a high-throughput approach to simultaneously study the effects of numerous gene perturbations in a single experiment. A recent development in CRISPR-based single-cell techniques incorporates a feature barcoding technology, making it possible to capture both the mRNA and gRNA from the same single cell. The feature barcoding technology involves the inclusion of a capture sequence on the single-cell gel beads, whose complement can be introduced into each gRNA. This allows the gRNA to be captured and eventually amplified prior to sequencing. However, with the technology in its early stages, it is not straightforward on how the different gRNA structural parameters affect gRNA capture and CRISPR efficiency. To overcome this, we tried different combinations of (i) capture sequence, (ii) capture sequence position and (iii) gRNA backbone to identify an optimal gRNA scaffold for CRISPR-activation gene perturbation studies. The screening is performed on human embryonic stem cells where we overexpressed two TFs, ASCL1 and SOX17. Here, we report our observations and provide recommendations for future CRISPR-based single-cell experiments.

Funding Source: Singapore National Research Foundation Competitive Research Programme (NRF-CRP20- 2017-0002)

Keywords: CRISPR activation, single-cell RNA sequencing, feature barcoding technology

Poster: 404

TRANSPLANTATION OF PERSONALIZED ARTIFICIAL BILE DUCT COVERED WITH CHOLANGIOCYTE FROM CHEMICALLY DERIVED HEPATIC PROGENITORS USING 3D BIOPRINTING

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Cholangiopathy caused by chronic progressive bile duct disorders has limited treatment options and cure possibilities. 3D bioprinting technique with bio-materials is emerging as a new approach for construction of functional tissues and 3D artificial organs. Here, we combined the bile duct scaffold manufactured by 3D bioprinting with hepatic progenitors to demonstrate safety and adequacy of 3D artificial bile duct (ABD) transplantation to a rabbit model. We previously reported that human primary hepatocytes (hPHs) are reprogrammed into human chemically derived hepatic progenitors (hCdHs) using small molecules A83-01, CHIR99021 and hepatocyte growth factor (HGF). We confirmed differentiation potential of hCdHs into cholangiocytes demonstrated by an increase the gene expression of cholangiocyte markers by qPCR and immunostaining. The 3D scaffold was made of two layers, the inner layer enhances the hCdHs arrangement, attachment and differentiation while the outer layer served to increase mechanical stability. Indeed, we validated that the 3D scaffold condition enhanced gene expression of cholangiocyte markers compared with 2D condition. Next, we manufactured personalized ABD of rabbit then, seeded hCdHs into the ABD in vitro and differentiated into cholangiocyte for 14 days. To further characterize our hCdHs derived cholangiocyte-like cells (hCdHs-Chols), we generated cholangiocyte-like cell organoids (CLC-organoids) for Rhodamin 123 assay and performed immunohistochemistry (IHC) of cholangiocyte markers CK7, CK19, CFTR and ZO-1. Finally, we transplanted the ABD into resected mid portion of common bile duct (CBD) to prove whether the ABD can stably and suitably replace with native rabbit bile duct (NBD), we performed complete blood count (CBC) and biochemical test in blood, IHC of ABD post-transplantation and ICG cholangiography in rabbit bile duct in vivo. As expected, the rabbits received ABD with hCdH-Chols had reduced liver damage marker expression (AST, ALT and ALP) as well as survived longer than control rabbits transplanted with the empty ABD. Thus, our method could contribute to clinical application as it can produce patient-specific artificial bile duct for transplantation. Furthermore, it could provide a scalable platform to treat broad spectrum of bile duct diseases.

Funding Source: This research was supported by grants from the National Research Foundation of Korea (NRF)no. 202100000210002.

Keywords: 3D bioprinting, Personalized artificial bile duct, Chemically derived hepatic progenitors

Poster: 405

CRISPR ACTIVATION DIRECTS DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO RETINAL PIGMENT EPITHELIAL CELLS

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Regenerative cell therapy is an exponentially growing field that aims to regenerate a lost function, cell type or tissue due to damage, ageing or disease. Transplantation of retinal pigment epithelial (RPE) sheets derived from human induced pluripotent cells (hiPSC) is a promising cell therapy for RPE degeneration, such as in age-related macular degeneration. Current RPE replacement therapies, however, face major challenges. They require a tedious manual process of selecting differentiated RPE from hiPSC-derived cells, and most importantly need replenishment of multiple expensive cytokines. In order to overcome the issues, we hypothesized that endogenous activation of key transcription factors will be sufficient to directly differentiate pluripotent stem cells into mature RPE tissue, without the need for costly growth factors and laborious protocol. Using the CRISPR-dCas9 mediated activation (CRISPRa) gene editing system, hiPSC IMR90, was activated to overexpress PAX6, MITF and OTX2, and subsequently directly differentiated into RPE cells. We show that multiplexed endogenous activation of all three genes resulted in pigmented, cobbled shaped foci

of CRISPRa induced RPE cells (CRISPRa-RPE) at day 40 with canonical RPE marker genes (such as PMEL, RPE65 and BEST) progressively upregulated over time. The RPE sheets were further purified simply by removal of the non-RPE cells under an inverted microscope. The purity of the CRISPRa-RPE population based on the PMEL17 expression was more than 96%. In addition, the CRISPRa-RPE sheet grown on transwell plate was stained for ZO-1 (tight junction marker) and was imaged under confocal microscopy to determine RPE cell boundaries. Further functional assessment by measuring TEER and photoreceptor phagocytosis assay identified the biological features of these RPE cells. This achievement will reduce the cost and time in producing mature RPE for retinal cell therapy.

Funding Source: Agency for Science Technology & Research

Keywords: CRISPR activation, RPE cells, differentiation

Poster: 406

IDENTIFICATION AND CHARACTERIZATION OF MARKER GENES TO PREDICT PROPENSITY FOR NEURAL PROGENITOR DIFFEREBTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELL LINES.

Kuroda, Takuya[1], Yasuda, Satoshi[1], Matsuyama, Satoko[1], Miura, Takumi[1], Sawada, Rumi[1], Matsuyama, Akifumi[2], Morioka, Masaki S.[3], Yamamoto, Yumiko[4], Kawaji, Hideya[3], Itoh, Masayoshi[5], Kawai, Jun[5], Sato, Yoji[1]

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Human induced pluripotent stem cells (hiPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, hiPSCs have been expected to provide new applications for regenerative medicine/cell therapy. Although various in vitro differentiation protocols have been developed for efficient derivation of specific cell types, hiPSC lines vary in their ability to differentiate into specific lineages. Therefore, surrogate markers that accurately predict the differentiation propensity of hiPSCs could be helpful for the development and manufacture of hiPSC-derived cells for therapies and in vitro assays. Here, we tried to identify the genes that potentially predict the differentiation propensity of hiPSCs into neural progenitor cells (NPCs). Using 10 hiPSC lines, we performed NPC-differentiation by two types of methods (suspension and adhesion culture) and examined expression of NPC marker genes with qRT-PCR analysis. Next, the comprehensive gene expression profiling of undifferentiated hiPSCs was applied to searching genes correlated significantly with the differentiation levels of hiPSC lines into NPCs. As a result, we identified 3 genes correlated commonly with differentiation propensity using both of the culture methods. We performed functional analysis of these genes with loss-of-function experiments introducing lentiviral shRNA-expressing vectors into hiPSCs for stable knockdown and finally confirmed their involvement in NPC differentiation. Our research will allow us to present a novel factor for defining the molecular mechanism underlying differentiation propensity of hiPSCs and to select the suitable cell lines for future translational applications.

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

Poster: 407

ANALYSIS OF MEMBRANE TRAFFICKING THROUGH RAB PROTEINS IN PARK8 IPSC-DERIVED NEURONS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder which causes tremor, rigidity, postural instability, bradykinesia, and non-motor symptoms. Mutations in LRRK2 gene are the most prevalent cause of autosomal dominant PD. Our group previously reported that induced pluripotent stem cells (iPSC) derived from a patient with I2020T mutation in LRRK2 (I2020T LRRK2-iPSC) in a Japanese family (the Sagamihara family) replicate the pathologic phenotype evident in the PD brain to some extent. Recently, the pathogenic mutant LRRK2 have been shown to enhance phosphorylation of Rab proteins, which is a related molecule to early or late or recycling endosome. In this study, to examine whether I2020T mutant LRRK2 affect intracellular membrane traffic through Rab proteins, we analyzed these protein levels and its intracellular localization using the differentiated neurons from I2020T LRRK2- or isogenic-iPSC. In addition, to evaluate the puncta of Rab endosome of iPSC-derived neurons, we used combined method of ImageJ and CellProfiler. At 21 days in vitro, we found that I2020T LRRK2 iPSC-derived neurons exhibited mislocalization of several Rab proteins compared to isogenic-iPSC-derived neurons. Furthermore, mitochondrial degeneration of I2020T LRRK2 iPSC-derived neurons tended to decrease. These results suggest that I2020T LRRK2 mutation may affect mitophagy and regulation of intracellular membrane trafficking in PD.

Keywords: iPS, Parkinson's disease, Rab

Poster: 408

NANOMEDIC VIRUS-LIKE PARTICLE DELIVERY SYSTEM ENABLES TRANSIENT TRANSDUCTION OF CRISPR BASE EDITOR TO INDUCE GENOMIC EXON SKIPPING

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CRISPR base editors enable the direct transition of single nucleotide at a targeted genomic locus without double stranded DNA brake. However, RNA off-target editing by the base editors may pose a risk for future base editing therapy, especially

when the base editors are delivered with AAV vector and expressed for long term. To this end, we thought to utilize the extracellular nanovesicle NanoMEDIC system, which utilize HIV Gag and viral envelope protein for delivering the Base editor into cells transiently. As a disease model, we constructed gRNA targeting splice acceptor site of exon 45 in human dystrophin gene, aiming to induce therapeutic exon skipping to restore truncated dystrophin protein. By utilizing a luciferase reporter vector in which dystrophin exon 45 is flanked by exons of luciferase gene, we investigated the exon skipping activity. When we packaged the base editor and gRNA into NanoMEDIC particle and inoculated into target HEK293T cells, we found that properly designed gRNA enables induction of exon skipping. We are currently investigating further optimization of the system and latest results will be discussed. We hope this nanovesicle-medicated delivery of base editor will be applied for in vivo exon skipping to treat Duchenne muscular dystrophy and beyond.

Keywords: CRISPR base editor, Virus-like particle delivery system, Exon skipping for Duchenne muscular dystrophy

Poster: 409

THERAPEUTIC EFFECT OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS ENCAPSULATED IN CELL FIBERS ON INFLAMMATORY BOWEL DISEASE MODEL IN MICE

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This presentation reports therapeutic effects on colitis of umbilical cord-derived mesenchymal stem cells (UC-MSCs) encapsulated in hydrogel microtubes known as “cell fiber”. Inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease are refractory gastrointestinal diseases with chronic intractable erosions or ulcers in the digestive tract. Since these diseases are often resistant to various existing treatments, a novel curative treatment with mucosal healing is required. Mesenchymal stem cells (MSCs) demonstrate high immunoregulatory functions and tissue regeneration ability through several mechanism, including secretion of paracrine factors, cell complementation, or their differentiation plasticity. We have previously shown that cell therapies using bone marrow or umbilical cord-derived MSCs effectively suppress the severity of colitis and promote the recovery from colitis in rodent models. However, most MSCs accumulated in the lungs when administered intravenously, resulting in their low mobilization in the intestinal tract, the target organ. In general, therapeutic efficacy is associated with cell distribution to the target organ, local cell therapy is expected to be highly effective by directly targeting the intestinal tissues. Meanwhile, directly administrated cells might be damaged significantly by intestinal bacteria or aggressive cytokines in vivo. To address this issue, we employed “cell fiber” technology, expecting the following two advantages; 1) MSCs cultured in cell fibers maintained a three-dimensional state similar to in vivo and high cell viability for a long time. 2) MSCs in the fibers locally administered near the intestinal tract are protected from a cytotoxic environment, while cell-derived factors secrete from the fibers and act on the target organ. In fact, higher concentrations of anti-inflammatory and tissue regeneration-related factors were observed in the culture supernatant of UC-MSC-fiber compared to those of 2D-cultured MSCs. Additionally, UC-MSC-fiber showed superior therapeutic effects in several colitis models. We will report on the mechanism of the therapeutic effects of the UC-MSC-fiber on colitis.

Funding Source: 1) Japan Agency for Medical Research and Development, 2) CellFiber Co., Ltd.

Keywords: Cell fibers, Umbilical cord-derived MSCs, Inflammatory bowel disease

Poster: 410

PRODUCTION OF A SINGLE-BATCH OF 15 BILLION HIPS CELLS WITHIN A 10L BIOREACTOR WITH AN EXPONENTIAL AMPLIFICATION FACTOR OF 276-FOLD WITHIN 6.59 DAYS

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2D cell culture has been widely used to manufacture the first generation of cell therapies. However, due to the drawbacks of scale-out process (footprint, workforce use, variability and subsequent QC expenses), the industry is shifting towards the goldstandard for bioproduction scale-up, i.e. bioreactors, with goal of addressing mass-markets with standardized and affordable products. The main format in bioreactor culture is aggregates. Aggregate culture has demonstrated a number of limitations, including scalability challenges, mostly due to mixing conditions and the associated shear stress, severely impacting yields and quality. So far, only a few teams have publicly shared results showing the successful cultivation of pluripotent stem cells in large-scale bioreactors. One notable recent achievement by Pigeau et al. (2020) is the production of a batch of 37 billion pluripotent stem cells in a 10L bioreactor in 6 days, with an amplification factor of 40-fold. Here using a new technology based on a high-speed cell encapsulation microfluidics, we report a 276-fold amplification of hiPSCs over 6.59 days in a 10L bioreactor with best-in-class cell viability and pluripotency. Also documenting the scale-independent amplification profile obtained with C-Stem in 30mL, 500mL, 1.5L and 10L bioreactors, we argue that the C-Stem™ technology is amenable to produce commercial-size batches of stem cells in larger bioreactors.

Keywords: Bioreactor, iPS, Pluripotent stem cell

Poster: 411

HIGH DENSITY BIOPROCESSING OF HUMAN PLURIPOTENT STEM CELLS BY METABOLIC CONTROL AND IN SILICO MODELING

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Human pluripotent stem cells (hPSCs) are a unique source for the production of functional human cell types, fueling advanced regenerative therapies. The envisioned applications will require the constant supply of billions of cells generated by robust and economically viable bioprocesses. We here combine instrumented stirred tank bioreactor (STBR) technology with in silico process modeling to take hPSC bioprocessing to the next level. Perfused suspension culture (3D) of matrix-free hPSC aggregates in STBRs was applied to identify and control process-limiting parameters including pH, dissolved oxygen, Glucose and Lactate levels, and the obviation of osmolality peaks. Media supplements promoted single cell-based process inoculation and hydrodynamic aggregate size control. Wet lab-derived process characteristics enabled predictive in silico modeling as a new rational for hPSC bioprocess development. As a result, long-term maintenance of exponential cell proliferation was enabled, thereby achieving a 70-fold expansion and an unmatched density of 35 x 106 cells/mL while reducing media requirements by 75%. This boost in process efficiency was applicable to all three genetically independent hPSC lines tested, despite the observation of cell-line specific culture properties such as aggregate size patterns. Furthermore, we have developed a systematic approach that enables maintenance of aggregate size distribution patterns across different STBR platforms and process scales. Consequently, when combined with feedback-based process control, the robust process upscaling from 150 mL to 250 mL and ultimately 500 mL scale was achieved in two different STBR platforms. Importantly, hPSCs generated by our conditions showed an unchanged, efficient differentiation potential into specific progenies derived from definitive endoderm, mesoderm and ectoderm. This highlights full preservation of the hPSCs' pluripotency and the achieved homogeneity of the cell quality resulting from our novel high density, large scale process. The study provides a deeper understanding of hPSC physiology, better definition of culture requirements, a straightforward strategy for the controlled production of hPSCs and their progenies, and the requirements for future process automation at industry-compliant conditions.

Keywords: high density hPSC cell culture, in silico process modeling, stirred tank bioreactor

Poster: 412

HIGH-THROUGHPUT POLYMER LIBRARY SCREENING TO OPTIMISE NAIVE HUMAN PLURIPOTENT STEM CELL RESETTING EFFICIENCY

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The recent reports of naive human pluripotent stem cell (hNPSC) generation and expansion, as well as their higher differentiation potential over their primed counterparts, has made hNPSCs very interesting to the stem cell community. However, the process of hNPSC generation is still complex; protocols typically require feeder cells, hypoxic conditions and lengthy optimisation. The resetting efficiency is often low, and some cell lines have shown resistance to reversion. In order for these cells to be used in a wide range of applications, their culture conditions need to be optimised; with undefined components, such as Matrigel or feeder cells, replaced. We have previously shown that a human serum-derived protein, Inter-a-inhibitor (IaI) can support the growth and long-term maintenance of hPSCs in vitro without inducing differentiation or genetic abnormalities. Unpublished data also shows IaI can also support hNPSC generation and expansion; by adding IaI to formulations for naïve resetting such as RSeT and 2iGöY media, hNPSCs can be obtained in coating-free, defined conditions. The cells show transcriptional and protein expression of naïve markers, overall DNA hypomethylation and increased mitochondrial oxidative respiration. One concern we identified was the resetting efficiency; our results showed a highly variable resetting efficiency, with KLF17+ hNPSC percentage at passage 1 ranging from 20 to 78%. To optimise the resetting process, we have used a high-throughput screening strategy to test a large library of synthetic polymers, using Nanog and KLF17 markers. Naïve resetting of hPSC lines was performed in feeder-free conditions, with the cells transferred to the polymer screening platform after the full resetting using PGXL/2iGöY. The cell phenotype data was then correlated with TOF-SIMS ion content data to investigate the chemical properties relating to hNPSC resetting. In this work, we investigate the use of synthetic polymers to reduce the chemical complexity of the substrates used in hNPSC resetting, to make a more robust method and take hNPSC culture to wider usage and applications. This new platform has the potential to generate simple, off-the-shelf products for hNPSC culture, as well as be incorporated in other biomaterial platforms to improve hNPSC attachment and survival.

Funding Source: The Wellcome Trust

Keywords: High throughput screening, Human Naive Pluripotency, Culture Optimisation

Poster: 413

AUTOMATED VISUAL QUALITY CONTROL OF IPSC COLONIES WITH DEEP LEARNING

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Treating diseases using gene therapy, cell therapy or engineered tissue, so called Advanced Therapy Medicinal Products (ATMP), is a rapidly growing field within life science, and the use of human pluripotent stem cells (iPSCs) for development of ATMPs carries great promise. With the development of ATMPs comes the growing need for accurate quality control (QC). Today, visual inspection of iPSCs under microscopes is an essential QC step and is dependent on well-trained human experts. However, human visual inspection is labor intense and involves a risk of unwanted subjectivity. To address this we have developed a deep learning algorithm to automatically identify iPSC colonies of aberrant morphological and textual characteristics. Current deep learning based methods are designed for the identification of iPSC colonies among background and other cell types with subsequent determination of colony quality. However, these are not applicable to industrial large-scale production of iPSC where cells are typically grown in a homogenous cell layer in flask culture. Thus, the novelty of our method is that it is developed for images with a uniform 2D layer of iPSCs instead of dispersed iPSC colonies surrounded with background and other cell types. The images we used in this work were derived from a commercial feeder-free, non-colony cell culture. A convolutional neural network (CNN) is a type of artificial neural network that is particularly suitable for analyzing images as it preserves spatial information. As these networks require large datasets, we have augmented images of a dataset to increase sample size and to achieve class balance between good and poor quality images to improve accuracy. The labels are based on the overall state of the culture. The CNN learns morphological and textural features to distinguish between good and poor quality cultures without feature engineering by a human expert. We were able to achieve a high validation accuracy of up to 90% and are optimizing the model for higher generalizability. This project has contributed to the evidence that visual QC of iPSC cultures can be more automated with deep learning methods. In future work, we intend to extend the algorithm to estimate the density of cells and further reduce subjectivity to advance automated QC in the manufacturing of iPSCs.

Keywords: Human induced pluripotent stem cells (hiPSC), Deep Learning, Quality Control

Poster: 414

TRANSCRIPTOMIC PROFILING OF HIPSCS GROWN IN NOVEL MICROFLUIDIC CELL CULTURE CHAMBERS

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Given the widespread need to generate homogenous cell populations, we sought to optimise a single-cell cloning workflow utilising novel microfluidic cell culture chambers and assess the effect of this approach on human induced pluripotent stem cells (hiPSCs). Exploiting interfacial tension, small-scale cell culture chambers are rapidly fabricated on polystyrene surfaces using only matrix coating and an immiscible translucent fluorocarbon overlay. Thus, chambers are sealed and separated from each other via fluid walls. Each chamber utilises less than 1 µl of cell culture medium to cultivate cells while remaining overlaid with the fluorocarbon, resulting in an increased surface-to-volume ratio compared to conventional culture plates. Using this novel culture approach, we established an optimised single-cell cloning workflow for hiPSCs with efficiencies up to 94% yielding genomically stable and pluripotency retaining clones. Within less than ten days, genome-edited homogenous cell populations, verifiably derived from a single cell, were obtained. We further assessed the applicability of the microfluidic approach for bulk culture of hiPSCs. Interestingly, transcriptome profiling of hiPSC populations grown in microfluidic chambers for several days revealed an increase in protein-coding genes particularly of the mitochondrial genome. This change was reversed upon transferring the cells back to conventional culture plates. We did not detect an increase in mitochondrial DNA copy number. Hence, the observed transient transcriptional changes may reflect a metabolic adaption to the culture environment of the small-scale microfluidic chambers. In summary, the novel microfluidic approach is highly compatible with single or bulk hiPSC culture workflows.

Keywords: RNA-Seq, single-cell cloning, microfluidics

Poster: 415

SEMI-AUTOMATED OPTIMIZED METHOD TO ISOLATE CRISPR/CAS9 EDITED HUMAN PLURIPOTENT STEM CELL CLONES

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CRISPR/Cas9 editing systems are currently used to generate mutations in a particular gene to mimic a genetic disorder in vitro. Such "Disease in a dish" model based on human pluripotent stem cells (hPSCs) offers the opportunity to have access to virtually all the cell types of the human body. However, the generation of such mutated hPSCs still remains fastidious. Current CRISPR/Cas9 editing approaches lead to a mixed cell population containing simultaneously edited and non-edited cells. These edited hPSCs needs therefore to be isolated through a dilution cloning approach that is time-consuming and tedious. Herein, we developed a semi-automated method for the clonal isolation of edited hPSCs. We used the CellCelector™ platform which combines automated single cell and colony picking system and 24-well cell culture plates featuring thousands of nanowells per well. Such nanowell plates allow cultivating of hundreds of individual clones per well in parallel after seeding of a hPSC single cell suspension. The method provides an image-verified proof of monoclonality. Once hPSC colonies have been grown in nanowells, those originating from single cells were automatically picked and transferred to standard cell culture plates for further expansion. We evaluated the effectiveness of single cell isolation using a mix of two different constitutively fluorescent cells. The analysis of independent expended colonies showed

that they were composed of unique fluorescence labelling thus demonstrating the purity of automated cell isolation. Then we optimized CRISPR/Cas9 editing conditions to knock out a particular gene (ALMS1). After electroporation or lipofection, hPSCs were seeded on nanowells for single cell isolation and colony formation. Genetic analysis of isolated colonies confirmed the effectiveness of clonal isolation while obtaining 100%-edited or completely unedited colonies. Finally, comparison with a manual isolation through limiting dilution cloning indicated that this new approach improved the clonal isolation and reduced the time required to produce a new edited hPSCs line. This new method of CRISPR/Cas9 editing and clonal isolation will greatly improve the generation of edited hPSCs for the study of genetic diseases.

Funding Source: I-Stem is part of the Biotherapies Institute for Rare Diseases supported by the Association Française contre les Myopathies - Téléthon

Keywords: Human pluripotent stem cells, CRISPR/Cas9, Clone isolation

Poster: 416

ALGINATE MICROCARRIERS AS LONG-TERM SCAFFOLDS FOR IPSCS EXPANSION IN SUSPENSION BIOREACTOR

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In its second project phase, the European Bank for induced pluripotent Stem Cells (EBiSC) aimed for long-term self-sustainability of the repository. The robustness of established high volume scalable protocols developed during the first project phase were tested on three different iPSC lines (derived from a healthy donor, a donor with cardiac disease, and a gene-edited tool line) to support the banking of the nearly 900 high quality iPSC lines and their derivatives available via the EBiSC catalogue. iPSCs were passaged in parallel in 2D (plates) and 3D formats (Matrigel-coated alginate microcarriers) across two different organisations. Inoculated alginate microcarriers, as a scalable, low shear stress and high nutrient diffusion culture system, were maintained in suspension in impeller free 50 mL tubes using the bench-top 3D cell culture incubator (CERO®). After four passages, cells were harvested for analysis and cryopreserved (n≥3, per condition per line). Full QC panel (flow cytometry, immunocytochemistry, qRT-PCR, trilineage differentiation, molecular karyotyping by SNP array, post-thaw viability and recovery, sterility and Mycoplasma tests) show sterile culture, comparable viability and a preservation of a typical pluripotent phenotype in 3D conditions. At both study sites and across all three iPSC lines, no cell line-specific optimisations were required, demonstrating that this upscaling approach can be used to reproducibly expand and maintain pluripotency of iPSCs from diverse backgrounds whilst also reducing medium and plasticware consumption.

Funding Source: This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 821362. JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

Keywords: alginate microcarriers, bioreactor, EBiSC

Poster: 417

DESIGN AND CONSTRUCTION OF A MULTI-LOADING DOCKING PLATFORM FOR MAMMALIAN CELLS

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Genome engineering of mammalian cells is currently limited by the lack of efficient methods for the fast, precise and stable integration of large amounts of heterologous DNA into specific genomic locations. The mechanism that we present here in a multiloading docking platform based on the use of two different site-specific recombinases phiC31 and Bxb1, and the piggyback and sleeping Beauty transposons. phiC31 is used during the construction of the platform. This recombinase is used by phages to establish the lysogenic life cycle. During integration, phiC31 drives recombination between the attP and the attB attachment sites on the phage and host genome, respectively. The end result is an integrated phage genome flanked by new attL and attR sites, each containing half sites derived from attP and attB. Under inducing conditions, the phage genome is excised via integrase-mediated recombination between attL and attR regenerating attP and attB attachment sites. This action is directed by phiC31 in the presence of an accessory protein (the recombination directionality factor, RDF). The alternative use of phiC31, alone or together with RDF, allows for the indefinite incorporation of docking platform units into the targeted locus. Each platform unit contains four independent integrations sites. The whole mechanism is made possible by the coordinated and alternative use of piggyback and sleeping Beauty transposons, that at each building cycle remove residual DNA fragments (plasmid sequences, selection markers, etc.,). Once the platform is in place, the Bxb1 recombinase is used to upload the different genes of interest (GOIs): markers, therapeutic genes or even complete regulatory routes. Different GOIs can be uploaded simultaneously or sequentially into the platform and, if more space is required, additional platform units could be added afterwards. The platform can be equipped with the desired promoters, either constitutive or inducible or, if preferred, with different types at the same time. In addition to this, the platform can be placed anywhere in the genome of any cell type.

Funding Source: PRD2018/87. General Directorate of Research. Balearic Government. PI18/00334. Carlos III Health Institute. RTC-2017-6696-1, (NANOGROW). Spanish Ministry of Science and Innovation

Keywords: Genome engineering, Multiloading docking platform, Protein Expression

Poster: 418

A ROBUST AND SEMI-AUTOMATED HIPSC GENE EDITING WORKFLOW

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Human induced pluripotent stem cells (hiPSCs) have the potential to address a broad range of research and clinical applications. However, the inability to robustly manipulate hiPSCs as single cells has, until recently, been a significant hurdle. We have recently shown the robustness and significant advantage of using the VIPS™ system in combination with MatriClone™ matrix (in solution) to achieve clonal outgrowth with 30-40% efficiency compared to under 10% achieved with limited dilution (LD). This significantly decreases total workflow time and cost by reducing the need for large quantities of plates or high reagent consumption. Critically, in contrast to typical LD techniques, the VIPS system ensures clonality by providing a "double-lock" of assurance, therefore establishing new standards in efficient single cell cloning. This study translates the improvements that the VIPS and MatriClone bring to a hiPSC gene-editing workflow. Using CRISPR-Cas9 via RNP delivery we aimed to disrupt the EMX1 gene locus in an hiPSC line. Following nucleofection, cells were single cell seeded via VIPS or limiting dilution (LD) and grown out in media containing MatriClone. Plates underwent daily whole-well imaging on the VIPS system to confirm clonality and track outgrowth of the colonies. As previously described, the VIPS demonstrated a 3-4 fold improvement in numbers of colonies successfully derived from single cells per plate when compared to the LD control. From the VIPS-seeded plates, 100 clones were selected and genotyped to confirm indel formation in the EMX1 locus. Three clones containing a confirmed indel were then expanded and underwent characterisation. Selected clones exhibited pluripotency marker expression of Oct4 and Tra-1-60 via ICC/IF and normal karyotype, validation that pluripotency and genomic integrity is maintained. Here we have demonstrated successful gene editing with VIPS using EMX1 as proof of concept. Importantly, these data show the advantages of using VIPS with MatriClone over traditional LD methods. By increasing efficiency, we estimate a 50% reduction in typical gene-editing workflows, providing a platform for improving low-efficiency editing projects. Moreover, the VIPS system provides quality data assurance and reporting that allows for real time assessment and regulatory compliance.

Keywords: Clonality, Cell Therapy, Gene Editing

Poster: 419

SPATIALLY DIRECTING STEM CELL FATE FOR OSTEOCHONDRAL TISSUE ENGINEERING

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The osteochondral interface between cartilage and bone enables effective transmission of stresses during articulation of joints. This is enabled by transitions in the composition and organization of the extracellular matrix, structural features that develop under the influence of morphogen gradients. Here, I will introduce our benchtop approach enabling biomaterials to be encoded with morphogen gradients for osteochondral tissue engineering. We use the principle of density-driven phase separation to generate tunable gradients that are then encapsulated by gelation or polymerization. This method, requiring only a mold and a micropipette injector, can be used to encode a range of gradients into common biomaterial systems. Using this approach, we cast gradients of bone morphogenetic protein 2 (BMP2) into gelatin methacryloyl (GelMA) hydrogels containing human mesenchymal stem cells. Osteochondral tissues were generated over 28 d, with the sustained release of BMP2 triggering local osteogenesis and mineralization at one end of the tissue construct. The use of a fluid redistribution strategy ensured the formation of a highly integrated zonal tissue, which avoided common issues with delamination or the exclusion of cells at the tissue interface. As well as potential suitability as a tissue graft, this system provided insight into the formation of the osteochondral tidemark, suggesting potential applications for modeling tissue development.

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Keywords: Osteochondral, Tissue Engineering, Growth Factors

Poster: 420

GENERATION OF A GENE-CORRECTED IPSC LINE OF MCARDLE DISEASE

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McArdle disease (glycogen storage disease type V) is a rare autosomal disorder caused by mutations in the PYGM gene, which encodes the muscle isoform of the glycogen phosphorylase. This leads to an inability to break down glycogen in the muscle cells, manifested in exercise intolerance, fatigue and myalgia. At this moment, there is no cure for this disorder and the current treatments are focused on the mitigation of the symptoms. The main aim of our investigation has been the generation of an iPSC model of McArdle disease and to stablish an isogenic matched gene-corrected control iPSC line using CRISPR-Cas9 technology. As starting material, we have used peripheral blood mononuclear cells (PBMCs) isolated from a McArdle patient carrying the second most frequent mutation in the Spanish population (c.2392T>C; p.W798R). For the reprogramming process a non-integrative methodology, which involves the use of Sendai viruses, has been employed. At this stage, we have confirmed the pluripotency and the quality of the generated iPSCs lines. Subsequently, patient-derived iPSCs where edited using an approach that involve ribonucleoprotein complexes (RNPs) to the delivery of the Cas9 along with an ssDNA oligonucleotide repair template to achieve the homology directed repair (HDR). RFLP assays were performed to test efficiency and Sanger sequencing was done to select the edited clones. Finally, the absence of undesirable off-target mutations was verified and clones where expanded and tested for pluripotency and quality markers. The generation of an iPSC McArdle model and its matched isogenic control will be very useful not only for modelling this disease but also to search for pharmacological and future cell therapies.

Funding Source: Fondo de Investigación Sanitaria, Instituto de Salud Carlos III cofunded by European Regional Development Funds (ERDF): PI15/00484 and PI18/00151 to MEGP.

Keywords: McArdle disease, CRISPR-Cas9, iPSC model

Poster: 421

HIGH-CONTENT, LABEL-FREE FUNCTIONAL IMAGING OF HUMAN IPSC-DERIVED NEURONAL CELL LINES BY MEANS OF HIGH-DENSITY MICROELECTRODE ARRAYS

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Recent advances in the field of cellular reprogramming have opened a route to studying the fundamental mechanisms underlying common neurological disorders. High-density microelectrode-arrays (HD-MEAs) provide unprecedented means for high-content electrical imaging of neurons at different scales, ranging from network through single-neuron to subcellular features. In this work, HD-MEAs are used in vitro to characterize and compare human induced-pluripotent-stem-cell-derived dopaminergic and motor neurons, including isogenic neuronal lines modeling Parkinson's disease and amyotrophic lateral sclerosis. Reproducible electrophysiological network, single-cell and subcellular metrics are used for phenotype characterization and drug testing. Metrics, such as burst shape and axonal velocity, enable the distinction of healthy and diseased neurons. The HD-MEA metrics can also be used to detect the effects of dosing the drug retigabine to human motor neurons. Finally, it is shown that the ability to detect drug effects and the observed culture-to-culture variability critically depend on the number of available recording electrodes.

Funding Source: European Research Council Advanced Grant. Grant Numbers: 694829, 875609 Innosuisse - Schweizerische Agentur für Innovationsförderung. Grant Number: 25933.2

Keywords: electrophysiology, high-density microelectrode arrays, induced pluripotent stem cells

Poster: 422

THE STEMCELLFACTORY: AUTOMATING REPROGRAMMING AND GENOME EDITING

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Human induced pluripotent stem cells (hiPSCs) offer unprecedented opportunities for disease modeling, drug screening and personalized therapy. In particular, hiPSC-derived cells have been increasingly used to study cellular and molecular pathomechanisms underlying diseases caused by specific genetic variants. CRISPR/Cas9-based genome editing has greatly facilitated the generation of distinct mutant hiPSC lines along with isogenic controls. The growing demand for genome-edited cells has created a strong need for automated systems that handle this complex process. To meet this need, we developed the StemCellFactory (SCF), a modular system for automated reprogramming of peripheral blood mononuclear cells (PBMCs) and CRISPR/Cas9-based genome editing of the resulting hiPSCs. Crucial components include a liquid handling unit, a density gradient-station for cell separation, an automated clone picker, high speed microscopy and an Amaxa 4D nucleofector with a 96-well shuttle™ which are all interconnected with a robotic arm to automated incubators, material hotels and analytical tools for cell culture quality control. For automated reprogramming, readily accessible peripheral blood samples were collected and induced to generate erythroid progenitor cells (EPCs) as well-defined starting cell population for reprogramming. A newly developed automated Percoll-based density gradient-station enables further EPC enrichment before transduction with reprogramming factor-encoding Sendai viruses. Primary hiPSC colonies formed with an average efficiency of 0.2 % and were automatically picked with a CellCelector module. Alongside the reprogramming pipeline, we developed an efficient, automated nucleofection procedure ensuring high hiPSC viability. Genome editing processes were validated with three independent hiPSC lines by employing ribonucleoprotein complexes consisting of Cas9 protein and a crRNA:tracrRNA duplex targeted against a candidate gene. Editing efficiencies were comparable to the manual process with indel rates of up to 90%. We expect this automated set-up to largely facilitate the generation of disease-specific and gene-corrected hiPSC lines for disease modelling and drug discovery.

Funding Source: The project "StemCellFactory III" is funded by the European Regional Development Fund (ERDF) under grant number EFRE-0800972.

Keywords: StemCellFactory, genome editing, automation

Poster: 423

A THERMOSENSITIVE CHITOSAN HYDROGEL-BASED 3D IN VITRO HUMAN NEURONAL CULTURE MODEL

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In vitro human brain models are fundamental to study cell interactions with other cells or with their surroundings in the central nervous system (CNS) and to study neuronal (dys) functions and connectivity. The advent of human induced pluripotent stem cells (iPSCs) presents opportunities to model brain human diseases, offering a platform for drug screening, in order to reduce the number of drug failures in clinical trials. Moreover, they are gaining increasing attention in the field of tissue engineering and regenerative medicine, for the possibility to be used as potential treatment for neurodegenerative diseases. In particular, the use of h-iPSCs cultured in three-dimensional (3D) systems better represents the complex in vivo tissue architecture, filling the gap between conventional 2D cell cultures and in vivo models, as they can offer insights into human-

specific functions without translation from animal to human physiology. Despite of this, the encapsulation of human derived neurons for the development of 3D neuronal networks is still limited. Over the last few years, increasing attention was focused on the development of in vitro neuronal models based on natural biomaterials. As the CNS tissue is extremely soft, hydrogels offer potential new strategies in this sense. For this purpose, in this study, a thermogelling-chitosan solution was developed, and the versatility of the thermosensitive chitosan-based scaffold as artificial matrix for 3D neuronal networks was investigated. Chitosan is well known for its biocompatibility, biodegradability, muco-adhesiveness, antibacterial activity and ability to perform sol-gel transition under specific external stimuli, also in the presence of cells. Chitosan thermogels were fabricated and characterized by different techniques. The scaffold was used to encapsulate human-induced neurons to carry out morphological characterization by immunofluorescence techniques and a preliminary electrophysiological characterization of spontaneous activity by Micro-Electrode Arrays. Results show that this 3D model can be considered a proper platform for neuronal in vitro studies. Furthermore, thanks to its injectability, the formulation is an excellent candidate for minimally invasive therapeutic cell delivery and as alternative ink for 3D bioprinting.

Funding Source: D. Di Lisa research activity is co-financed by Programma Operativo Por FSE Regione Liguria 2014-2020, n. RLOF18ASSRIC".

Keywords: Chitosan, human-induce pluripotent stem cells, 3D neuronal in vitro platform.

00:00 - 1:00 EDT

POSTER SESSION 4

TISSUE STEM CELLS AND REGENERATION

Poster: 233

THE EFFECTS OF FIR-PRECONDITIONING ON SURVIVAL OF RAT BONE MARROW-DERIVED STEM CELLS ASSOCIATED WITH MITF-AKT-MTOR-EXOSOME MANUFACTURE

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A previous study from our laboratory observed the protective effects of far-infrared irradiation (FIR) on bone marrow-derived stem cells (BMSCs) against oxidative stress. However, it remains unknown precisely how FIR influences BMSC survival. We identify an unexpected route among the expression of MITF, BCL2, mTOR, and exosome in FIR-preconditioned BMSCs. MITF siRNA demonstrated that loss of MITF expression not only inhibited cell proliferation but also reduced the FIR-mediated expression of mTOR, BCL-2, and exosome. mTOR signaling pathways have been implicated in cell growth, proliferation, and survival. We also found that rapamycin, a potent and selective inhibitor of mTOR, when combined with MITF siRNA, repressed FIR-mediated CD63 and BCL-2 expression. In addition, FIR-preconditioned BMSCs demonstrated more tolerance in multiple stressful environments than untreated BMSCs. The elevated exosomes in conditioned medium derived from FIR-preconditioned BMSCs also repaired H9c2 cells that sustained cellular damage after multiple stressful conditions. Taken together, these results support the idea that FIR-preconditioned BMSCs and its conditioned media could contribute to boosting cell survival against multiple stress conditions and offers a more unified mechanism of MITF-Akt-mTOR associated with exosome manufacture. FIR preconditioning has far-reaching implications for improving the cellular basis of BMSC-related regenerative medicine therapy.

Funding Source: NRF-2016R1A6A3A11933448; 2020R1I1A1A0105459511; 2017R1A-6A03015562

Keywords: far-infrared irradiation, bone marrow derived mesenchyma stromal cells, MITF

Poster: 432

THE USE OF MESENCHYMAL STEM CELLS IN ANIMAL MODELS OF ANASTOMOTIC LEAK: A SYSTEMATIC REVIEW

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Anastomotic leak is the most feared complication of gastrointestinal (GI) surgery, leading to significant morbidity and mortality. In the UK, approximately 50,000 anastomoses are performed annually for colorectal disease alone, with leak rates as high as 19%. Various strategies have been tried, but failed to produce any significant positive effect. Mesenchymal stem cell (MSC) technology is used clinically in various tissue regenerative applications to promote wound healing. The safety and efficacy of these multipotent cells on anastomotic wound healing has yet to be defined. This review aims to investigate whether mesenchymal stem cells confer any benefit when applied to animal models of GI anastomotic leak, identify what methodology is used to deliver MSCs to the anastomotic site and how efficacy is assessed. A systematic review was performed searching the MEDLINE, EMBASE, Web of Science, Cochrane Library and clinicaltrials.gov databases between 01/01/1947 – 01/05/2020. All studies that explored the delivery of mesenchymal stem cells to a GI anastomosis in an animal model were considered. The search returned 1483 articles. 1205 abstracts were screened leaving 17 articles for full assessment. 12 studies met the inclusion criteria and reported outcomes on a total of 438 gastrointestinal anastomoses were constructed in 4 different animal species in 11 models of anastomotic leak. Seven were performed in rat colon models. No studies used an animal model with a known leak rate. Significant variance was observed in histological outcomes with efficacy of MSC application demonstrated in five out of the twelve studies with one study demonstrating a benefit in leak rate. Median ARRIVE guideline compliance across all twelve studies was 50.0% (IQR 45.7-62.5). Colorectal models of anastomotic leak had a greater compliance, 60.8% (IQR 63.2-64.5) compared to non-colorectal studies 45.4% (IQR 43.8-49.0). Delivery of MSCs to a GI anastomosis in an animal model is safe and feasible. The application of MSCs may confer benefit but findings are currently limited to surrogate histological outcomes. There is consistency in outcome measures reported but variance in how

they are measured. Poor compliance to ARRIVE guidelines but good compliance to current international consensus in lower GI anastomotic leak models was observed.

Keywords: Anastomosis, Mesenchymal Stem Cells, Leak

Poster: 433

IMPACT OF TAMOXIFEN THERAPY ON HUMAN ADIPOSE-DERIVED STEM CELLS AND IMPLICATIONS IN ADIPOSE TISSUE ENGINEERING FOR BREAST RECONSTRUCTION

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Adipose-derived stem cells (ADSCs) are actively being investigated for reconstructive purposes, and particularly in breast reconstruction for post-mastectomy breast cancer patients. Tamoxifen, a selective oestrogen receptor modulator is an adjuvant therapy for oestrogen receptor positive breast cancer in pre-menopausal patients. The effects of such therapies on ADSCs is poorly understood and may impact successful and efficient ADSC based regenerative strategies. Here we investigated the effects of Tamoxifen's active metabolites - Afimoxifene (4-Hydroxy-Tamoxifen), Endoxifen (N-desmethyl-4-hydroxytamoxifen) and their combination (Tamoxifen) on the ADSC survival and functionality. We report that the ADSCs tolerated doses of up to 1μM drug with no decline in cell viability or increase in apoptosis. ADSCs showed no functional decline in adipogenic differentiation or gene expression at physiologically relevant doses but had a downward trend at higher concentration. VEGF165 protein expression was also not significantly impacted at physiologically relevant doses but showed an insignificant decline at high drug concentration. Overall, at physiologically relevant doses, Tamoxifen treatment did not show any deleterious effect on the ADSC survival and functionality and their use in breast cancer management is unlikely to negatively impact ADSC based breast reconstruction strategies.

Funding Source: National Breast Cancer Research Institute (NBCRI), Ireland

Keywords: Adipose-derived stem cells, Breast reconstruction, Tamoxifen therapy

Poster: 434

DIABETIC MICROENVIRONMENT DETERIORATES THE REGENERATIVE CAPACITIES OF HUMAN ADIPOSE TISSUE-DERIVED PERICYTES

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It has been previously reported that the diabetic microenvironment could impair the normal physiological functions of adult stem cells. Hyperglycemia is associated with altered physiology of the pericytes (PCs) in the diabetic patients. The loss of PCs causes abnormal endothelial cell behavior and increases the risk of vascular complications in diabetic patients. In this study we aim to investigate the effect of diabetic microenvironment on the regenerative capacities of human adipose tissue derived PCs. Human adipose tissue-derived PCs were cultured in PC-specific cell culture medium supplemented with either human normal serum (NS) or diabetic serum (DS) for 6, 14 and 30 days. PCs were assessed for morphological and ultrastructural characteristics, angiogenic differentiation potential, DNA repair and inflammatory markers expression. Our data shows altered PC morphology, flattened cells with dull edges when cultured in DS compared to healthy, well defined cells in NS. When examined by transmission electron microscopy for ultrastructure, cells cultured in diabetic serum showed thicker nuclear membrane and more fragmented mitochondria and their surface topography showed more invaginations compared to the cells cultured in normal serum. In diabetic serum, the angiogenic differentiation potential of the human PCs decreased as confirmed by tube formation assay and VEGF-A gene expression. Apoptosis increased as assessed by Annexin-PI staining and confirmed by high expression of Perlman A and Clic4 proteins. However, the level of intracellular ROS showed no significant difference between the two groups. DS caused PCs to express DNA repair genes as SIRT1, TERF1 and TERF2, and showed upregulated expression of both proinflamatory and anti-inflammatory genes including ICAM1, IL-6, and TNF-α. We conclude that the diabetic microenvironment results in decreased regenerative potential of human adipose tissue derived PCs. This may contribute to the vascular complications presents in the patients of Type-2 diabetes mellitus (T2DM).

Funding Source: STDF 5300 ASRT 7304

Keywords: Type II diabetes mellitus, Human adipose tissue -derived pericytes, Stem cells

Poster: 435

ENHANCED ADIPOSE DERIVED MESENCHYMAL STEM CELL DIFFERENTIATION INTO OSTEOBLASTS BY PHOTOBIOMODULATION

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The most severe chronic bone metabolic disease in humans is osteoporosis. Currently, Adipose Derived Mesenchymal Stem Cells (ADMSCs), when used in stem cell regenerative therapy, has shown remarkable potential treating osteoporosis disease. Photobiomodulation (PBM) has sparked interest internationally because of its ability to proliferate stem cells and facilitate the induction of differentiation. The use of growth factors and PBM combined has demonstrated enhanced proliferation and differentiation of ADMSCs into osteoblast cell lines. This in vitro study combined the use of osteogenic differentiation inducers and PBM at visible and near-infrared (NIR) wavelengths between 400 - 1100 nm using a single fluence to determine the proliferation and differentiation effectivity of ADMSCs into osteoblasts. The cells were characterised using both early and late osteoblast protein markers identified via the use of flow cytometry, immunofluorescence, spectroscopy and morphology. Biochemical analysis investigated cell morphology, viability, proliferation, cytotoxicity, Mitochondrial Membrane Potential, Reactive Oxygen Species and their migration rate. The genetic expression of transcription factors, signalling proteins and miRNAs were determined using ELISA. The successful outcome of this in-vitro study will provide relevant scientific knowledge

and a standardization for osteogenic differentiation. Additionally, this study may reach clinical trials for use in the treatment of osteo-degenerative diseases like osteoporosis.

Funding Source: This work is supported by the SARChI/NRF-DST (Grant No. 98337), received by Daniella Da Silva and Prof Heidi Abrahamse. Dr Anine Crous was supported by the NRF S&F Scarce Skills Postdoctoral Fellowship (Grant no: 120752).

Keywords: Adipose derived stem cell differentiation, Osteoblasts, Photobiomodulation;

Poster: 436

ENHANCING MOUSE EMBRYONIC STEM CELLS CARDIAC DIFFERENTIATION BY KNOCKING-DOWN DAND5 GENE

Inacio, Jose Manuel[1], Lopes, João von Gilsa[1], Silva, Ana M[1], Cristo, Fernando[1], Marques, Sara[1], Futschik, Matthias E[2], Belo, Jose A[1]

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Heart failure due to cardiomyocyte and cardiovascular diseases are the leading cause of mortality in developing countries. Identifying and studying the developmental and cellular pathways involved in heart formation could inspire novel regenerative treatments. Here, we show the potential of a single molecule, DAND5, in mouse pluripotent stem cell-derived cardiomyocytes specification and proliferation. Dand5 loss-of-function generated more than double cardiac beating foci compared to the wild-type cells. We observed that both the early formation of cardiac progenitor cells and the increased proliferative capacity of Dand5 KO mESC-derived cardiomyocytes contribute to this higher number of derived cardiac cells. Moreover, a transcriptional profiling sequencing and a quantitative RT-PCR analysis showed that early cardiac gene networks governing cardiomyocyte differentiation, cell cycling, and cardiac regenerative pathways were upregulated, and concomitantly the levels of genes involved in cardiomyocyte maturation were reduced. These findings prompt DAND5 as an unexplored driver for the generation and expansion of pluripotent stem cell-derived cardiomyocytes for further clinical application.

Funding Source: This work was supported by Fundação para a Ciência e a Tecnologia (PTDC/ BIM-MED/3363/2014) and Scientific Employment Stimulus to JMI (Norma Transitória 8189/2018), and iNOVA4Health -UID/Multi/04462/2013, PT2020.

Keywords: Cardiac Differentiation, Dand5, Cardiomyocyte Proliferation

Poster: 438

LENTIVIRAL VECTOR BASED IN VIVO GENE THERAPY UNCOVERS THE CONTRIBUTION OF DIFFERENT HEPATOCYTES TO POST-NATAL LIVER GROWTH AND HOMEOSTASIS IN MICE

Milani, Michela[1], Starinieri, Francesco[2], Canepari, Cesare[2], Simoni, Chiara[1], Plati, Tiziana[1], Biffi, Mauro[1], Russo, Fabio[1], Cammarota, Eugenia[3], Naldini, Luigi[1], Cantore, Alessio[1]

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Liver-directed gene therapy has shown successful results in adults with hemophilia. However, broadening its application to pediatric patients is currently challenging. To address this issue, we took advantage of integrating lentiviral vectors (LV) and showed stable transgene expression in the liver of adult mice, dogs and non-human primates, after systemic intravenous (i.v.) delivery. Here, we investigate the efficiency of LV liver gene therapy in growing mice and the possible contribution of different hepatocyte subpopulations to liver growth and homeostasis. We treated mice at different ages, 1 day-old (newborn), 2 week-old (juvenile) or 8 week-old (adult) by i.v. injection of LV expressing coagulation factor IX (FIX). We observed the highest transgene output in juvenile mice, followed by newborn and the lowest in adult treated mice. We measured the percentage of transgene-positive liver area and, despite the higher transgene output, we did not detect differences between newborn and juvenile treated mice, while the transduced area in adult mice was 4-times lower. Moreover, adult-treated mice showed a periportal transduction bias that started to appear in juvenile treated mice and was absent in newborn mice, suggesting that the biology underlying this outcome is established during liver growth. All treated animals showed long-term maintenance of transgene expression, suggesting that hepatocytes belonging to different zones can all contribute to homeostasis. We then investigated clonal proliferation of hepatocytes in Alb-Cre/Rosa26-Confetti mice up to 1 year of age. Interestingly, most of hepatocytes appeared quiescent, with only 25% of them generating continuously growing clusters, covering 90% of liver area at 6 weeks of age. Taken together, these data show that timing of LV administration impacts on gene therapy efficacy and that LV marking allows identifying and tracking hepatocyte subpopulations that contribute differentially to liver growth and turnover. Ongoing studies based on ultrastructural imaging of sinusoidal lining and single-cell omics of hepatocytes will uncover the bases of these findings. Our work provides a rationale for the application of LV-mediated liver gene therapy to pediatric patients and shed light on mechanisms of liver tissue dynamics.

Keywords: Liver growth, Liver gene therapy, Lentiviral vectors

Poster: 439

MOUSE BONE MARROW ENDOTHELIAL CELLS HOLD BROAD POLYCLONAL POTENTIAL TO REGENERATE BONE MARROW VASCULARITY

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The bone marrow endothelial cells (BM-ECs) are the key part of bone marrow niche critical for regulation of hematopoiesis. The rapid regeneration of endothelial network in BM after conditioning-induced injury determines the success of hematopoietic cell transplantation. However, the cellular mechanisms of BM-ECs regeneration remain unclear. Our aim was to understand how the BM-ECs regenerate at the single cell and clonal level. First, we performed single cell RNA sequencing (scRNA-seq) of mouse BM-ECs and combined our data with published scRNA-seq datasets. The metanalysis included 13,037 BM-ECs and revealed that sinusoidal ECs express high levels of Fcgr2b, but are negative for Ly6c, while the arterial type ECs express Ly6c, but no Fcgr2b. We also identified transition cells that were double positive for Fcgr2b and Ly6c (DP-ECs). Analysis of possible differentiation trajectories based on RNA velocity algorithms suggested that the DP-ECs give rise to both sinusoids and arteriolar cells. Imaging of the BM niche revealed that DP-ECs localize between the sinusoidal and arterial ECs, linking the distinct ECs within the vascular network. Next, using FACS we prospectively isolated sinusoidal, arterial and DP-ECs from BM and developed single cell-derived EC organoid assay to study their clonogenic potential. In contrast to in silico predictions, single sorted ECs from all fractions formed multicellular vessel organoids on BM stromal monolayer with similarly high efficiency in limiting dilution assay (1/4.3 single sorted cells), indicating the broad regeneration potential of BM-ECs regardless of their phenotype. Finally, we used Cdh5-CreER-Rainbow mice to study clonality of BM-ECs regeneration 7 and 21 days after conditioning with 9.5 Gy irradiation and BM transplantation. We observed highly polyclonal pattern of BM vasculature after irradiation, without any detectable oligoclonal regions. Modeling based on local assortativity and machine learning supports that the observed multicolor fate mapping indicates contribution of many BM-ECs, rather than rare progenitor fraction, to vascular regeneration. Concluding, our novel single-cell clonogenic assay and multicolor fate mapping indicate broad and polyclonal contribution of BM-ECs to regeneration of BM vascularization.

Funding Source: This work was supported by the Ministry of Science and Higher Education (DI2019 010249), National Science Center (2018/30/A/NZ3/00495) and Foundation for Polish Science (POIR.04.04.00-00-5F16/18-00)

Keywords: bone marrow niche, endothelial cells, bone marrow transplantation

Poster: 440

REGULATORY MECHANISM OF DENTAL EPITHELIAL STEM BY MICROENVIRONMENTAL OXYGEN-RHOA-YAP/TAZ SIGNAL IN MOUSE INCISOR

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Molecular oxygen (O2) is an important component of the stem cell niche microenvironment, but little is known about how O2 governs epithelial stem cell (ESC) behavior. In this study, we demonstrate that O2 plays a crucial role in regulating the proliferation of ESCs using the continuously growing mouse incisors. We have revealed that slowcycling cells in the niche are maintained under relatively hypoxic conditions compared with actively proliferating cells, based on the blood vessel distribution and metabolic status. Mechanistically, we have demonstrated that, during hypoxia, HIF1α upregulation activates the RhoA signal, thereby promoting cortical actomyosin and stabilizing the adherens junction complex, including merlin. This leads to the cytoplasmic retention of YAP/TAZ to attenuate cell proliferation. These results shed light on the biological significance of blood-vessel geometry and the signaling mechanism through microenvironmental O2 to orchestrate ESC behavior, providing a novel molecular basis for the microenvironmental O2-mediated stem cell regulation during tissue development and renewal.

Keywords: Oxygen, epithelial stem cells, RhoA-YAP/TAZ

Poster: 441

HSA-MIR-143-3P INHIBITS MAPK SIGNALING IN HUMAN CORNEAL EPITHELIAL STEM CELLS

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The corneal epithelium covers the anterior surface of the eye and its homeostasis is maintained by the corneal epithelial stem cells (CESCs) residing in the basal layer of the limbus at the corneo-scleral junction. The molecular mechanisms governing the maintenance of these stem cells are not clear so far, since there is no specific marker for their identification and owing to the use of heterogeneous population of stem cells along with the transient amplifying cells. To overcome this problem of heterogeneity, in this study the CESCs were enriched to 80% by a two-step protocol i) isolation of basal limbal epithelial cells by differential enzymatic treatment ii) followed by Laser capture micro dissection of cells with nucleus to cytoplasm ratio ≥ 0.7. Small sequencing with this enriched CESCs identified six microRNAs (hsa-miR-3168, hsa-miR-21-5p, hsa-miR-143-3p, hsa-miR-150-5p, hsa-miR-1910-5p and hsa-miR-10a-5p) were

identified to be highly expressed in enriched CESCs compared to central corneal epithelial cells (CCECs). The sequencing data was validated by quantitative real time PCR (qPCR) and locked nucleic acid in-situ hybridization (LNA-ISH). Further to understand the regulatory role of hsa-miR-143-3p miRNA transfection studies were carried out in human primary limbal epithelial cells. The high expression of hsa-miR-143-3p increased the colony forming potential (10.04 ± 1.35 %, p<0.001) with the ability to form holoclones in comparison to inhibitor treated (0.27 ± 0.24%, p<0.001) and control (3.33 ± 0.71%). Further, at the transcriptional and translational level, with the increased expression of this miRNA, the cells showed increased the expression of (i) stem cell markers (ABCG2, ΔNP63, NANOG, OCT4 and KLF4) and reduced expression of (ii) differentiation marker (Cx43), (iii) MAPK signaling regulators (p-ERK1/2, p-p38,p-c-JUN,p-c-FOS, p-ATF2 and p-p53) and (iv) its targets (DVL3, KRAS, MAPK1 and MAPK14). Downregulation of MAPK signaling regulators indicated the inhibition of MAPK signaling by hsa-miR 143-3p. Based on the above observations, regulatory role of hsa-miR-143-3p in maintenance of stemness through inhibition of MAPK signaling pathways was thus elucidated.

Funding Source: Department of Biotechnology (India) provided funds for the work, CSIR (India) provided Senior Research Fellowship and Commonwealth Scholarship Commission UK provided Scholarship to Lavanya Kalaimani.

Keywords: microRNA, MAPK signaling, stemness

Poster: 442

DEFINING DIVERSITY AND SIMILARITY OF EPITHELIAL STEM CELL POPULATIONS AND THEIR ANATOMICAL ENVIRONMENT IN MURINE SKIN AND ORAL EPITHELIUM

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Skin and oral epithelium provide a barrier against physical stress and infection and are continuously regenerated by resident epithelial stem cells. Mouse tail epidermis harbors slow- and actively-cycling stem cell populations corresponding to specialized tissue architectures, interscale and scale, respectively. In human skin and mouse oral epithelium, there is a unique undulation structure called rete ridges and dermal papillae. It remains elusive, however, whether epithelial stem cells in different tissues have any common or tissue-specific features in relation to tissue architecture; and how stem cell patterns are regulated by tissue structures responding to environmental changes. Here we analyze stem cell dynamics, anatomical structure, and transcriptome profiles in the murine oral and skin epithelium. By combining H2B-GFP pulse-chase analysis and lineage tracing with Dlx1-CreER and Slc1a3-CreER markers, we find that slow- and actively-cycling stem cells in oral epithelium lie in a specific anatomical location relative to the rete ridges and regenerate their own territory during homeostasis. The RNA-sequencing analysis provides common gene signatures of slow- and actively-cycling populations between skin and oral epithelium and identifies possible markers for capturing stem cell heterogeneity in vivo and in vitro. Our work provides cellular atlas and molecular basis of stem cell heterogeneity in epithelial tissues, which will lead to the future in-depth studies of stem cell-niche interactions as well as applications in regenerative medicine.

Keywords: Epithelial stem cells, Oral epithelium, Stem cell dynamics

Poster: 443

DISTINCT TYPES OF STEM CELL DIVISIONS REGULATE HAIR FOLLICLE ORGAN REGENERATION AND AGING

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Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thinning and loss. Here we report that hair follicle stem cells (HFSCs) lose their regenerative capabilities during aging owing to the adoption of an atypical cell division program. Cell fate tracing and cell division axis analyses revealed that while HFSCs in young mice undergo typical symmetric and asymmetric cell divisions to regenerate hair follicles, upon aging or stress, they adopt an atypical 'stress-responsive' type of asymmetric cell division. This type of division is accompanied by the destabilization of hemidesmosomal protein COL17A1 and cell polarity protein aPKCλ and generates terminally differentiating epidermal cells instead of regenerating the hair follicle niche. With the repetition of these atypical divisions, HFSCs detach from the basal membrane causing their exhaustion, elimination and organ aging. The experimentally induced stabilization of COL17A1 rescued organ homeostasis through aPKCλ stabilization. These results demonstrate that distinct stem cell division programs may govern tissue and organ aging.

Keywords: Stem cell divisions, hair follicle stem cells, aging

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IDENTIFICATION OF NOVEL MOLECULAR PATHWAYS INVOLVED IN THE STEMNESS OF HUMAN GASTRIC CANCER

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Cancer stem cells (CSCs) exhibit unique characteristics of plasticity, unlimited self-renewal and quiescent state that make them responsible for tumor origin and heterogeneity, therapy resistance, recurrence and metastatic spread. In this

context, our group and others have demonstrated that SOX9, a developmental transcription factor that is a master regulator controlling super-enhancer dynamics in stem cells plasticity, regulates CSCs and exerts a relevant oncogenic role in different types of cancer such as glioblastoma, gastric, colorectal or pancreatic cancer. However, the molecular mediators and/or co-operators of SOX9 in the regulation of CSCs, which represent promising targets in cancer, remain poorly understood. Therefore, this work addressed this aspect, with a particular emphasis on gastric cancer (GC). In order to identify potential drivers of CSCs, we have accomplished biocomputational analyses using publicly available data of gastric cancer patients to identify the genes whose expression is most positively correlated with the expression of SOX9 in gastric cancer and selected a group of candidates based on their clinical impact. For the selected genes, we performed gain-and-loss of function studies in vitro and in vivo and omics strategies to evaluate their role in the regulation of CSCs and the processes linked to this population. Among our initial panel of candidate genes, we found that the expression of HMGA1, FGFR4, EHF, ECT2, TPX2, DIAPH3, KIF11, KIAA1804 and IL17RB decreased in SOX9-silenced cells and paralleled SOX9 expression in critical aspects of GC, such as Helicobacter pylori activity and/or cisplatin resistance, indicating that they can be relevant in the disease. Moreover, we went further in the study of DIAPH3 and KIF11, finding that their genetic silencing in GC cells severely impaired cell proliferation and oncosphere formation, and induced cell senescence and apoptosis in vitro, as well as reduced tumor growth in vivo. Following our approach, we identified KIF11 and DIAPH3 as critical oncogenic genes that play a relevant role in the stemness of GC and could represent suitable molecular targets for the treatment of the disease.

Keywords: stemness, cancer stem cells, gastric cancer

Poster: 445

THE INFLUENCE OF GOLD NANO SENSITIZER PHOTODYNAMIC THERAPY ON THE INVASIVE AND MIGRATORY ABILITIES OF LUNG CANCER STEM CELLS

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Despite advances in therapy, lung cancer is the leading cause of cancer mortality globally in both sexes. Cancer relapse and post-treatment dissemination suggest the presence of a subset of cancer cells that are not completely eradicated by existing therapies. These drug resistant populations of cells are called cancer stem cells (CSCs). Cancer metastases and the risk of secondary tumours are the most frequent causes of mortality in many cases. One important feature of metastases is the invasive ability of the cells, which is driven primarily by cell motility. Considering CSC proliferation and migration associated with metastases, a minimally invasive cancer treatment that has been widely accepted and used to treat a variety of malignancies including lung cancer such as Photodynamic therapy (PDT), along with the use of a nano drug carrier was used in this study. PDT is based on the principle of light stimulation of a photosensitizing drug that induces tumour cell death. Nano mediated PDT using gold nanoparticles have been seen to induce cell death in lung CSCs. In this study various physiological experiments including ATP proliferation, cell cycle analysis, ECmatrix cell invasion assay and cell migration were conducted to determine whether PDT using a gold nanosensitizer prevents CSC migration and invasion. Possible outcomes will include the inhibition of CSC migration and invasion, cell cycle arrest and decreased CSC proliferative abilities elaborating on the effectivity of nano mediated PDT treatment of lung cancer.

Funding Source: This research is supported by the NRF S&F -Scarce Skills Postdoctoral Fellowship (Grant no: 120752) received by Dr. Anine Crous; and the SARChI/NRF-DST (Grant no: 98337) received by Prof Heidi Abrahamse.

Keywords: Gold Nano Sensitizer, Photodynamic Therapy, Lung Cancer Stem Cells

Poster: 446

AGE- ASSOCIATED MECHANICAL CHANGES IN THE MURINE SKIN DRIVE CIRCADIAN CLOCK GENE REGULATION

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Aging can be defined as a decline or loss of adaptation during the increase of age, caused by a decay of functions and integrity. This has been shown to occur in tissues such as the skin. The skin is affected structurally and functionally during aging, although changes in the mechanical properties of the dermis are the most predominant. These changes include an increase in stiffness and a decrease in elasticity in the extracellular matrix (ECM). This shift in the mechanical properties is detected by the Hippo pathway transducers YAP1 and TAZ, which control the expression of specific gene expression patterns crucial for epidermal homeostasis. Epidermal cells require rhythmic daily oscillations to maintain homeostasis, which are controlled by the circadian clock. The clock controls tissue- and cell type-specific rhythmic genes, were Bmal1 is one of the main core clock transcription factors. This homeostatic control in the skin includes the oscillatory regulation of ECM components, which is also affected during aging. All this highlights a relationship between mechanical changes undergone during aging and the clock. We are analyzing how the mechanical changes occurring in the skin during aging affect the circadian clock. We have focused on how the age-related ECM stiffness changes affect the epidermal progenitors clock output genes. For this, we have addressed the connection between YAP1 and TAZ transducers with the core clock gene Bmal1. This will give new insights into how tightly circadian clocks and mechanical changes are regulated, and how they partly define the age-associated changes occurring in the epidermis.

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Keywords: Aging, Circadian rhythms, ECM

Poster: 447

ROLES AND RESPONSIBILITIES IN STEM CELL RESEARCH: A FOCUS GROUP STUDY WITH RESEARCHERS AND PATIENTS

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There have been fruitful efforts in identifying socio-ethical challenges of induced Pluripotent Stem Cell research (iPSC-research) in both the research and the clinical translation phase. Ethical challenges are broad, encompassing for example the procurement of donor material, questions about its ownership, side-effects, and costs of therapy. In both academia and policy-making there is an ongoing discussion about how to deal with these and other socio-ethical challenges. However, in relation to iPSC-research, little is known about the perspectives of researchers and patients regarding the ethical challenges of iPSC-research and how these should be dealt with. These stakeholders could offer a helpful insiders' perspective about how responsibilities are distributed in practice, how each party perceives their own role and that of others, and whether they anticipate possible obstacles. Therefore, we have conducted a qualitative study involving three focus group interviews with the first group consisting out of early till midlevel career stem cell researchers, the second group of late career stem cell researchers and the third group consisting of chronic lower back pain patients. The transcriptions of the focus groups have been thematically analyzed by using Nvivo 12. This paper discusses the focus groups' findings. The early till midlevel career researchers mentioned the difficulty of changing their work environment, possible problems of disseminating research to public, that both ethics and regulation could hamper research and that they would like more guidance and training in regulatory and ethical affairs. The late-career researchers mentioned, among others, that funding organizations play an important role in the direction and possibilities of research and they emphasize the importance of the interaction with patients. The patients focus group emphasized the importance of being involved early in research in order to inform researchers about the positive impact research could have and the importance of being informed properly, including understandable language, accessibility and expectation management. The paper will conclude by outlining how the findings could help to identify possible pitfalls, difficulties and possibilities for enhancing responsible iPSC-research.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825925.

Keywords: Ethics, Focus groups, Responsible research

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INTEGRATION OF CULTURED HUMAN TRABECULAR MESHWORK (TM) STEM CELLS TO TM IN A CELL LOSS HUMAN ANTERIOR SEGMENT ORGAN CULTURE (HOCAS) MODEL

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Trabecular meshwork (TM), a tiny porous tissue located in the irido-corneal angle of the eye is responsible for regulating intraocular pressure (IOP). Previous reports from our lab identified that the reduction in TM stem cell (TMSC) content with aging to be significantly associated with TM cell loss. The reduction of TMSCs was much higher in glaucomatous condition wherein there is a drastic reduction in total TM cells. This study aims to evaluate the efficacy of in vitro expanded TMSCs for TM regeneration in a cell loss human organ culture anterior segment (HOCAS) model. The human TM cells were isolated from donor eyes by collagenase digestion and cultured Stem Cell Growth Media (SCGM). Immunostaining identified that the SC (cells expressing p75 and high ABCG2) content to be 65.6±6.68% in primary culture which reduced to 12-14% on passaging. The efficacy of SCGM to maintain stemness was confirmed by the higher sphere forming efficacy (0.18%). A TM cell loss model to mimic glaucomatous condition was established in HOCAS with 0.002% saponin. After 24 hours of treatment, 26.97±0.35% cells in the filtering region were found to be TUNEL positive while the non-filtering region was negative. The cells in the non-filtering region expressed the neural crest derived SC marker-p75 but were negative for the universal stem cell marker ABCG2. The total TM cell content in filtering and non-filtering region reduced drastically after 1 week of saponin treatment. The IOP increased to 28.27±13.96 after saponin treatment from baseline pressure 15.64±5.26. Correspondingly, the outflow also decreased after saponin treatment. Transplantation of Qtracker labelled TM cells cultured in SCGM (3x105 cells p1-12.63% TMSCs, 87.3% TM cells) was carried out 24 hours after saponin treatment. After 3 days, both TM and TMSCs in the transplanted population were identified to be integrated in both filtering and non-filtering regions of TM. The transplanted cells did not induce any apoptotic response in the host tissue. Confocal analysis revealed the transplanted cells express ABCG2 and p75 proteins. Further studies are being carried out to elucidate the efficacy of the transplanted cells in restoring normal IOP.

Funding Source: SCIENCE AND ENGINEERING RESEARCH BOARD(EMR/2016/002351)

Keywords: Trabecular meshwork, Cell loss model, Transplantation

Poster: 449

DEVELOPMENT OF A NOVEL 3D PLATFORM TO GENERATE RETINAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS AT SCALE AND UNDER XENO-FREE CONDITION

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Irreversible retinal degenerative diseases leading to photoreceptor cell death and loss of sight are the leading cause of untreatable blindness with a huge impact on the life of affected individuals and imposing a substantial burden to healthcare providers. Currently there are no clinical treatments that restore lost photoreceptors and visual function and the efficacy of the few current therapies are limited to alleviating the symptoms, or slowing down the progression of disease. Considerable advances over the last decade in subretinal cell delivery and stem cell technology have increased expectations that retinal repair by cell transplantation could become a feasible clinical treatment. Therefore, development of new organoid platforms for generation and study of human retinal tissues is of utmost importance to provide retinal tissue for development of cell replacement for late-stage degenerative disease, and also for in vitro modelling of human retinal degenerative disorders and drug screening and molecular therapy testing to prevent photoreceptor degeneration. To this end, various protocols have been developed to generate retinal organoids from human pluripotent stem cells (using embryonic stem cells, ESC, and induced pluripotent stem cells, iPSC). However, existing protocols are labour intensive and rely on undefined animal-derived supplements, limiting their clinical application. Therefore, in this project we have developed an improved differentiation platform to generate rod and cone photoreceptor-enriched retinal organoids to provide new photoreceptor cells for cell replacement therapy. We applied novel strategies to generate the 3D retinal organoids from human pluripotent stem cells under xeno-free conditions and at scale. Analysis of organoid generation in the new platform using immunostaining and quantitative polymerase chain reaction (qPCR) showed they recapitulate aspects of neural retinal development including sequential expression of retinal markers and formation of laminated retinal tissue containing both rod and cone photoreceptors with segment-like structures. This platform offers a potential solution for the large-scale generation of photoreceptors under xeno-free and GMP-ready conditions for cell replacement therapy.

Funding Source: This research is generously funded by National Institute of Health Research (NIHR) Great Ormond Street (GOSH) Biomedical Research Centre (BRC) and Medical Research Council (MRC).

Keywords: Retinal Organoid, Human Pluripotent Stem Cells, 3D Culture

Poster: 450

ESTABLISHMENT OF HUMAN IPS-DERIVED CD68+ ALVEOLAR MACROPHAGE-LIKE CELLS FOR LUNG TOXICITY TEST

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Alveolar macrophages (AMφs), tissue-resident macrophages in the alveoli, play critical a role in the innate immune response against viruses, pathogenic microbes, inhaled toxic chemicals, and particular matters. Application of AMφs in vitro models of human lung toxicity test is huddled due to limited source of AMφs. Recent studies have reported that AMφs are originated from yolk-sac or fetal liver and not from circulating bone marrow derived-monocytes. Here, we differentiated human iPS cells into CD68+AMφ-like cells using a unique differentiation strategy. hiPS-AMφs showed the macrophage-like morphology and expressed AMφ-specific surface markers. Furthermore, they differentiated into M1 or M2 type of AMφs depends on the stimuli and phagocytosed E.coli. Altogether, hiPS-AMφs were mature and functional. These data suggest that hiPS-AMφs could be a useful source for developing in vitro model for pulmonary toxicity test.

Funding Source: This work was supported by Korea Environment Industry & Technology Institute (KEITI) through the Environment Health Action Program, funded by Korea Ministry of Environment (MOE) (2018001360003).

Keywords: Human alveolar macrophage, Phagocytosis, Human pluripotency stem cell

Poster: 451

DIFFERENTIAL HEMATOPOIETIC SUPPORT ACROSS THE ADIPOCYTIC DIFFERENTIATION AXIS: PHARMACOLOGICAL MODULATION TO ACCELERATE HEMATOPOIETIC RECOVERY

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The hematopoietic stem cell (HSC) niche constitutes a complex bone marrow (BM) microenvironment that tightly controls HSC proliferation. Adipocytes have been shown to inhibit hematopoietic progenitor proliferation, but adiponectin-expressing cells are required for full HSC support and pre-adipocytes can efficiently support the growth of hematopoietic cells in culture. Given that specific bone marrow stromal cell (BMSC) subpopulations, preadipocytes, and adipocytes are part of the same differentiation axis, this apparently contradictory data suggests that the same precursor can generate cells with divergent hematopoietic-supportive capacity. We thus hypothesized that pharmacological modulation of the adipocytic differentiation axis could inhibit bone marrow-derived adipocyte maturation and predict hematopoietic progenitor support. To test this hypothesis, we performed devised high content phenotypic screening pipeline to identify modulators of adipocytic differentiation with increased hematopoietic support in the absence of exogenous cytokines. For in vivo validation of the screen, compounds were tested for their capacity to shorten aplasia post lethal irradiation and bone marrow transplantation. Specifically, the Prestwick Chemical Library of FDA-approved drugs, a library containing

natural products and the library of chemicals generated from swiss academic laboratories were screened for their potential to prevent lipidation. After confirmation screen and dose-response determination, 49 inhibitors were selected. RNA-seq further classified compounds inhibiting adipocyte-specific gene expression as well as maintaining expression of hematopoiesis-supportive cytokines. Functional in vitro assays further identified a specific vitamin D analog as the strongest niche modulator, and transcriptome analysis revealed a CAR-like phenotype. In vivo administration significantly decreased both the adipocyte size and content of bones at day 18 post BM transplantation, increased platelet recovery and increased total number of CFU per leg. Thus, our pipeline allowed for the screening of more than 4000 compounds and identified a vitamin D derivative as a first candidate to induce a CAR-like phenotype and accelerate hematopoietic recovery in radio/chemotherapy-induced aplasia.

Funding Source: FS is funded by an SNSF grant number: 323530_183986, ON was funded for this project by SNSF grant PP00P3_183725, as well as EPFL and UNIL discretionary funds.

Keywords: Hematopoiesis, Microenvironment, Adipocytes

Poster: 452

INTEGRATED SINGLE CELL ANALYSIS IDENTIFIES UNIQUE MOLECULAR AND FUNCTIONAL FEATURES OF EXTRAMEDULLARY HAEMATOPOIESIS IN HUMANS

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In adults, most hematopoietic stem and progenitor cells (HSPCs) reside within the bone marrow (BM), giving rise to all mature blood cells. Yet at any given time, a small proportion of HSPCs circulates in peripheral blood (PB), and under severe stress and disease, also the spleen can significantly contribute to blood production. However, the cellular, molecular and functional composition of circulating and extramedullary HSPC pools remains unexplored. Here we discuss the single cell characterisation of the adult human HSPC pool found in the two main extramedullary haematopoietic tissues, spleen and PB, comparing it to BM. Using matched and unmatched samples from deceased and living donors, we profiled over 115,000 single CD34+ HSPCs by scRNA-seq and/or CITE-Seq and 3,900 single phenotypic haematopoietic stem cells / multipotent progenitors (HSC/MPPs) in functional assays. We find largely distinct HSPC compositions between tissues. The topography of the hematopoietic hierarchy in BM supports continuous HSPC proliferation and blood production. In contrast, modelling active differentiation using scRNA-seq data and flow cytometry analysis detect few to no proliferating progenitor cells in extramedullary sites. The balance of progenitors is heavily shifted from late progenitors in BM to early progenitors in spleen and PB. This indicates a cellular configuration in extramedullary tissues positioned for lineage-primed demand-adapted haematopoiesis. Importantly, the vast majority of HSC/MPPs in spleen and PB are molecularly distinct from those in BM, differing in adhesion molecules and lineage priming properties. Finally, single cell functional assays demonstrate that steady-state non-mobilised PB is dominated by quiescent HSC-like cells functionally restricted to erythrocyte and megakaryocyte production. The functional bias of PB HSC/MPPs towards a largely erythro-megakaryocyte skewed lineage output becomes imbalanced with age and in haematological conditions. In summary, the cellular and functional composition of HSPCs circulating in PB is uniquely different from that of BM, but faithfully report impairments of haematopoiesis. Our study thus identifies extramedullary cellular reservoirs for demand-adapted haematopoiesis and provides a framework of clinical relevance.

Keywords: Haematopoietic stem cells, Molecular and functional single cell analysis, Extramedullary haeamtopoiesis

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DRUGS TARGETING COLORECTAL CANCER STEM CELLS

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Introduction: 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. Methods: 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. Results: 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. Conclusion: 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.

Keywords: Cancer stem cells, Colorectal cancer, Markers

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HEMATOPOIETIC STEM CELLS AND HELPER T CELLS DIRECTLY INTERACT IN AN ANTIGEN-DEPENDENT MANNER, BIDIRECTIONALY MODULATING STEM AND T CELL FUNCTIONS

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Hematopoietic stem cells (HSCs) continuously generate blood and immune cells, including T cells and antigen-presenting cells (APCs). Antigen presentation by APCs to CD4+ helper T cells constitutes the central process in the orchestration of adaptive immune responses. While T cells play a vital role in regulating stem cell biology, stem cells are not considered to actively modulate the adaptive immune system. However, we have challenged that hypothesis and now we have evidence of antigen presentation by HSCs to helper T cells occurring. This interaction efficiently activates T cells and polarizes them towards an immunoregulatory state. Moreover, transient antigen presentation triggers a rapid cell cycle induction and differentiation of HSCs upon interaction with antigen-specific T cells, whereas chronic and sustained antigen presentation resulted in irreversible stem cell exhaustion. In malignant hematopoiesis, antigen presentation was associated with a stem-like state and poor clinical outcome. Our data reveal a bidirectional interaction between stem cells and the adaptive immune system, demonstrating that normal and malignant HSCs are not only passive receivers of immunological signals, but active modulators of their microenvironment.

Keywords: Immune Microenvironment, Hematopoietic Stem Cells, T cells

Poster: 455

REDUCED ESR1 EXPRESSION IN MUSCLE STEM CELLS AT THE CONCAVE SIDE ATTRIBUTES TO ADOLESCENT IDIOPATHIC SCOLIOSIS

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Adolescent idiopathic scoliosis (AIS) is one of the common pediatric musculoskeletal diseases. It occurs in disproportionately high percentage in females. Abnormal myogenesis may be a cause of AIS. However, the links between muscle defects and AIS has not been characterized. Here, we find that the expression level of estrogen receptor 1 (ESR1) decreases in Pax7+/MyoD+ myoblasts at the concave side in a subpopulation of AIS patients. Myoblasts isolated from the concave side display impaired differentiation ability. ESR1 is required for myoblast differentiation by activating Akt signaling. Reduced ESR1 activity in myoblasts leads to differentiation defects. When ESR1 activity is imbalanced at the para-spinal muscle, scoliosis is induced in mice. Furthermore, reactivation of ESR1 by raloxifene at the concave side alleviates the progress of scoliosis. This work reveals the inactivation of ESR1 signaling in paraspinal muscle is the cause for a subpopulation of AIS patients and new treatment strategy could be developed by targeting ESR1 signaling.

Keywords: Adolescent idiopathic scoliosis, Human myoblasts, Estrogen receptor 1

Poster: 456

STANDARDIZATION OF THE HUMANIZED OSSICLE TECHNOLOGY AS A PLATFORM TO MODEL LEUKEMIA AND SOLID TUMOR METASTASIS

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The engineering of humanized ossicles (hOss) recently emerged as a promising technology capable of overcoming some of the mouse model limitations. These hOss are miniaturized bone organs offering the in vivo reconstitution of the human mesenchymal niche and supporting the robust engraftment of a wide variety of human hematopoietic malignancies and bone metastases. The formation of hOss remains highly variable and largely dependent on selected primary human mesenchymal stromal cells (MSCs) exhibiting good proliferation and differentiation capacity. So far, this lack of reproducibility together with the diversity of existing protocols have precluded the broad adoption of the approach by the scientific community. Here, we report the standardized generation of hOss by exploitation of a custom-designed human mesenchymal cell line. The in vitro chondrogenic priming of these cells results in the generation of human cartilage, effectively remodeling into mature bone/bone marrow tissues upon ectopic implantation in mice by recapitulating the developmental process of endochondral ossification. Strikingly, our human mesenchymal cell line offers the reconstitution of a complex humanized bone marrow environment supporting the long-term engraftment of human cord blood hematopoietic stem and progenitor cells with balanced lymphoid and myeloid differentiation. Moreover, as compared to mouse bones our hOss model enables a superior and reproducible engraftment of primary acute myeloid leukemia samples, as well as breast cancer and patient-derived neuroblastoma cell lines. Taken together, our hOss approach represents a powerful tool towards deciphering the role of the human mesenchymal niche both in healthy but also in various pathologic settings. Beyond standardization, the use of mesenchymal cell lines also provides a malleable platform for the functional interrogation of factors involved in disease initiation and progression.

Keywords: Humanized Ossicle, Mesenchymal Cell Line, Leukemia, Solid Tumor Metastasis

Poster: 457

LINEAGE REPROGRAMMING TOWARD INDUCED NEURAL STEM CELLS WITH RE-ENGINEERED SOX17

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The rodents cannot represent all human phenotypes and genotypes comprehensively. Therefore, we need authentic human cell models which mimic the human body environment in vitro. Induced neural stem cells (iNSCs) are promising cell sources for regenerative medicine and neural disease modeling. The transdifferentiation to iNSCs lacks consensus protocols and suffers from low efficiency and reproducibility. Artificially evolved and enhanced transcription factors (eTFs) can be identified by rational protein engineering techniques and directed molecular evolution in mammalian cells. We have identified a variant of the endodermal factor SOX17 (eSOX17) that transdifferentiates embryonic, adult and old mouse fibroblasts into self-renewing and tri-potent iNSCs at high efficiency and speed. eSOX17 effectively works in four, three, or two-factor cocktails as part of mono as wells as polycistronic cassettes. Wild-type SOX2 and SOX17 fail to support the transdifferentiation to iNSCs in our system. Moreover, in the human system, we have directly transdifferentiated human iPSC-derived fibroblasts towards iNSC with eSOX17. Then, to take one step closer to neuromuscular disease modeling, spinal cord motor neurons are generated from human iNSCs by overexpression of ISL1 and LHX3. Also, these motor neurons are co-cultured with non-neuronal cells, for example, astrocytes. We will discuss the identification and characterization of eSOX17 and derived cell types and our efforts to apply it in the human system and differentiate them to specific neuronal subtypes.

Keywords: induced neural stem cells, transdifferentiation, directed molecular evolution

Poster: 458

ACENTROSOMAL MICROTUBULE ASSEMBLY IN QUIESCENT NEURAL STEM CELLS

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The ability of stem cells to switch between quiescence and proliferation is crucial for tissue homeostasis and regeneration. Drosophila quiescent neural stem cells (NSCs) extend a primary cellular protrusion from the cell body prior to their reactivation. However, the structure and function of this protrusion are not well established. Here, we show that in quiescent NSCs, microtubules are predominantly acentrosomal and oriented plus-end-out toward the tip of the primary protrusion. We have identified Mini Spindles (Msps)/XMAP215 as a key microtubule regulator in quiescent NSCs that governs NSC reactivation via regulating acentrosomal microtubule growth and orientation. We show that quiescent NSCs form membrane contact with the neuropil and E-cadherin, a cell adhesion molecule, localizes to these NSC-neuropil junctions. Msps promote NSC cell cycle re-entry and target E-cadherin to NSC-neuropil contact during NSC reactivation. Therefore, the neuropil can function as a new niche controlling NSC reactivation, which may be a general paradigm in mammalian systems.

Keywords: Drosophila, neural stem cells, quiescence, acentrosomal microtubule, reactivation, Msps, E-cadherin, neuropil, protrusion

Poster: 459

HIGH MITOCHONDRIAL MASS IDENTIFIES A SUB-POPULATION OF VEMURAFENIB-RESISTANT CANCER STEM CELLS IN MELANOMA

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Drug resistance still represents the main reason for therapy failure in melanoma patients. In this regard, cancer stem cells (CSCs) are thought to be responsible for treatment evasion and tumor relapse. Our hypothesis is that drug resistance in melanoma CSCs is partly driven by enhanced mitochondrial function. Here, we used A375 human melanoma cells to dissect the role of mitochondrial metabolism in conferring the CSC phenotype. More specifically, we employed fluorescent staining with MitoTracker to metabolically fractionate this cell line into mito-high and mito-low sub-populations by flow cytometry. Interestingly, cells with high mitochondrial mass (mito-high) were specifically enriched in the CSC marker ABCG2. Large cell size is another feature of the stem cell phenotype; herein, we observed a significant increase in mitochondrial mass in large cells, relative to the smaller cell population. Importantly, CSC-enriched melanospheres obtained from A375 cell line also showed the co-enrichment of ABCG2 and mitochondrial mass. Most significantly, we demonstrated that vemurafenib-resistant A375 cells (A375R), generated by growing the parental cell line in increasing concentrations of the drug, exhibited higher mitochondrial mass than their drug-sensitive counterpart. In particular, an increase in mitochondrial function (OXPHOS protein levels), biogenesis (PGC1-α protein levels) and fusion (OPA1 and MFN2 protein levels) was found in both A375 melanospheres and A375R cells with respect to A375 cell line. In summary, increased mitochondrial mass is associated with a stem-like phenotype and vemurafenib resistance in melanoma, suggesting that therapeutically targeting the mito-high CSC population might overcome drug resistance.

Funding Source: F. F. was supported by an AIRC fellowship for Italy.

Keywords: Melanoma, Mitochondria, Vemurafenib resistance

Poster: 460

HIPPO COMPONENTS YAP AND LATS1 REGULATE TROPHOBLAST STEM CELL SELF-RENEWAL AND GIANT CELL DIFFERENTIATION

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Intriguing reports on Hippo during the first cell fate specification prompted our investigation on Hippo dynamics during trophoblast stem cell (TSC) differentiation. Herein, we show a dynamic nucleo-cytoplasmic shuttling of the terminal Hippo component YAP during differentiation of murine blastocyst derived TSCs. Our results illustrated that YAP is predominantly in the nucleus of TSCs. We revealed a novel interaction between nuclear YAP and CDX2, an indispensable transcription factor for TSC maintenance. Using a series of deletion mutants, we identified a specific WW2 domain in YAP which can directly interact with PPQY motif in CDX2. Ectopic overexpression of YAP limited whereas diminution of endogenous YAP potentiated the CDX2 target, CyclinD1 levels. Further, loss-of and gain-in YAP function established the sequestration of CDX2 by YAP as a plausible mechanism to check excessive proliferation as evidenced by compromised BrdU incorporation in presence of excess YAP. Elevated pYAPSer127 levels of YAP in trophoblast giant cells (TGCs) indicated cytoplasmic retention of YAP in TGCs. In addition, a physical association between the core Hippo kinase LATS1 and LIMK2 in trophoblast cells and heightened LATS1-LIMK2 complex was detected in TSCs. Moderate level of LATS1 along with an upsurge of LIMK2 and pLIMK2Thr505 leading to enhanced phosphorylation of its target gene non-muscle Cofilin occurs in TGCs. Precocious overexpression of LATS1 during trophoblast differentiation led to decreased Prl2c2, a TGC genetic marker and diminished pLIMK2Thr505 and inactive cofilin (pCofSer3). Reduced pCofSer3 upon LATS1 overexpression is independent of chronophin, a phosphatase responsible for the removal of the phosphate group confirming our data on LIMK2 mediated regulation of cofilin by the Hippo component LATS1. LATS1 overexpression inhibited trophoblast endoreduplication as evidenced by TGCs with smaller sized nuclei associated with a lower ploidy level and disintegrated actin filaments. Taken together, our findings bring forward a multi-layered regulation of trophoblast self-renewal and differentiation by the Hippo components.

Funding Source: This work was supported by grants from Indian Council of Medical Research (2020-3442/SCR/Adhoc-BMS) to RA

Keywords: Trophoblast stem cells, Hippo, Trophoblast giant cells

Poster: 461

ANTI-VIRAL EFFECTS OF MIRNAS IN EXTRACELLULAR VESICLES OF MSCS AGAINST SARS-COV-2

Moon, Jisook[1], Park, Jae Hyun[2], Choi, Yuri[1], Lim, Chul-Woo[1], Park, Ji-min[1], Kim, Jae Ho[1], Hwang, Jin Woo[1], Choi, Moonbeom[1], Kim, Chul[1]

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), with no treatment, is an RNA virus that induces COVID-19. miRNA is an important regulator of gene expression that functions by inhibiting mRNA. Extracellular vesicles (EVs), with its ability to deliver miRNAs to recipient cells and regulate cellular conditions, is an interesting material. In this study, we identified miRNAs thought to play an important role in the biological function of virus-infected host cells, and examined the antiviral effects of miRNAs delivered by placental MSC-EV. There are several advantages of developing a therapeutic agent for SARS-CoV-2 using miRNAs in MSC-EVs. It is expected that miRNAs of MSC-EVs specifically bind to the 3'UTR of the SARS-CoV-2 genome, resulting in little side effects. Also, Since the 3'UTR sequence of the virus has few mutations and is highly conserved, it can be used universally in RNA viruses that have undergone mutations in the process of viral RNA replication. Various cargoes present in MSC-EVs act as very unique treatments with various effects, showing damaged tissue regeneration and immune regulation.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (NRF-2019M3A9H1103765)

Keywords: SARS-CoV-2, COVID-19, miRNA, Mesenchymal stem cell, Extracellular vesicle, 3'UTR

Poster: 463

MECHANISMS OF GASTRIC PROGENITOR CELL DIFFERENTIATION IN STOMACH EPITHELIAL HOMEOSTASIS

Takada, Hitomi[1], Sasagawa, Yohei[2], Yoshimura, Mika[2], Tanaka, Kaori[2], Nikaido, Itoshi[2], Kurisaki, Akira[1]

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The glandular stomach is anatomically divided into two compartments, the corpus and the antrum, and the lumen of both compartments is lined with a monolayer of epithelial cells arranged into invaginated gastric units. Those gastric epithelial cells are continuously turned over throughout life to maintain tissue homeostasis. This process is sustained by a population of highly proliferative progenitor cells that are localized in the isthmus region of gastric units. In the corpus, the isthmus progenitor cells are found in the upper-middle part of gastric units. Those progenitor cells differentiate into multiple epithelial cell lineages including pit cells, neck cells, parietal cells, chief cells, and endocrine cells, as they undergo a bidirectional migration to the luminal surface and the gland base. Although it is well known that the tight coordination of stem cell self-renewal and differentiation is critical for tissue integrity, the molecular mechanisms underlying the gastric progenitor cell fate decision during tissue homeostasis remain poorly understood. Here we took advantage of a high-throughput single-cell RNA-sequencing method, Quartz- Seq2, to dissect the gene expression profiles of the cells that comprise the gastric units. In this presentation, we will show the detailed results of in silico prediction of putative regulatory pathways responsible for gastric progenitor cell differentiation.

Keywords: Stomach, Single-cell transcriptome, Gastric progenitor cells

12:00 - 13:00 EDT

POSTER SESSION 5

CELLULAR IDENTITY

Poster: 501

CHRONIC SENESCENCE IN PRIMARY HUMAN DERMAL FIBROBLASTS MAY LIMIT THEIR REPROGRAMMING EFFICIENCY TO GENERATE INDUCED PLURIPOTENT STEM CELLS

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Dermal fibroblasts contribute to wound healing and are an appropriate cell source to generate induced pluripotent stem cells (iPSCs) by reprogramming. Age and different stressors or even the reprogramming factors themselves may induce senescence in fibroblasts limiting their reprogramming efficiency. Cellular senescence is characterized by a restriction of longevity and can prematurely be initiated in other cell types by the alkylating agent sulfur mustard (SM). In this study, we investigated the SM sensitivity and the induction of chronic senescence in primary human dermal fibroblasts (HDF) as a model to determine mechanisms potentially reducing their reprogramming efficiency in the future. HDF were treated with SM at final concentrations from 0.03 µM to 1000 µM or solvent control for 24 h and the XTT assay was used to determine the 50 % lethal concentration (LC50). Sub-lethal concentrations were used to investigate the induction of senescence in HDF by SM. Cells were exposed to 3 – 65 µM SM or 300 – 500 µM H2O2 and senescence-associated β-galactosidase (SA-β-gal) was stained histochemically over 31 days. The treatment of HDF with SM revealed a LC50 of 161.73 µM ± 7.31 µM. Using the former defined sub-lethal conditions, a time and concentration dependent senescence induction by SM and H2O2 was verified. Single doses of 24 µM, 40 µM or 65 µM SM resulted in stable senescence after 10 – 14 days. Senescent cells showed an increased cell size. HDF exposure to single, sub-lethal SM concentrations results in chronic senescence. HDF dysfunction may contribute to the chronic cutaneous wound healing disorder after SM exposure. The identification of this pathomechanism after SM exposure should be considered when regarding the cellular reprogramming of HDF to iPSCs since chronic senescence may prevent them from reprogramming. Future studies may unravel alternative therapeutic targets to improve reprogramming efficiencies for HDF, which may also be applicable for HDF from aged or stressed sources.

Keywords: human dermal fibroblasts, chronic senescence, reprogramming efficiency

Poster: 502

CONVERGENT DIFFERENTIATION: CORONARY BLOOD VESSELS FROM DISTINCT ORIGINS EVOLVE INTO EQUIVALENT TRANSCRIPTIONAL AND BEHAVIORAL STATES DURING DEVELOPMENT

Phansalkar, Ragini[1], D'Amato, Gaetano[2], Krieger, Josephine[2], Sinha, Rahul[3], Winn, Virginia[4], Zhao, Mingming[5], Red-Horse, Kristy[2]

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Most cell fate trajectories during development follow a diverging, tree-like branching pattern, but the opposite can occur when distinct progenitors contribute to the same cell type. In these instances of convergent differentiation, it is unknown if cells "remember" their origins by retaining transcriptional signatures from their progenitors or whether this could influence cell behavior. One such example occurs in the coronary vasculature—the blood vessels that supply the heart. Both the endocardium (Endo) and the sinus venosus (SV) produce arterial, capillary and venous cardiac endothelial cells (ECs), but whether these mature cells have transcriptional or functional differences related to origin is unknown. We addressed this by combining lineage tracing with single-cell RNA sequencing in embryonic and adult mice. At e12.5, shortly after coronary development begins, we found that capillary ECs transcriptionally segregate into two populations that have different lineage contributions and retain either SV- or Endo-specific gene expression. At e17.5, when the coronary vasculature is well-established but still undergoing remodeling, we found that ECs again segregate into two capillary populations. However, these had less correlation with lineage and no correlation with progenitor-specific transcriptional patterns. Instead, the genes differentially expressed at this stage were indicative of functional differences, including flow and hypoxia responses, and were correlated with location within the heart. Data from adults revealed a homogenous population of capillary ECs devoid of strong indications of either lineage or location. In agreement with this, we observed similar responses of SV- and Endo-derived ECs to cardiac injury. Finally, we provide evidence that human coronary development follows similar principles since single cell comparative analyses identified cell states closely matching mouse. From these data, we conclude that over the course of development, transcriptional heterogeneity in coronary ECs is first influenced by lineage, then by location, until heterogeneity disappears in the static adult heart. These results highlight the plasticity of ECs during development in response to changing cues, as well as the validity of the mouse heart as a model for human coronary development.

Funding Source: American Heart Association Predoctoral Fellowship

Keywords: convergent differentiation, lineage tracing, heart development

Poster: 503

TRAJECTORY RECONSTRUCTION IDENTIFIES DYSREGULATION OF PERINATAL MATURATION PROGRAMS IN MOUSE AND HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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A primary limitation in the clinical application of pluripotent stem cell derived cardiomyocytes (PSC-CMs) is the failure of these cells to achieve full functional maturity. In vivo, cardiomyocytes undergo numerous adaptive changes during perinatal maturation. By contrast, PSC-CMs fail to fully undergo these developmental processes, instead remaining arrested at an embryonic stage of maturation. To date, however, the precise mechanisms by which directed differentiation differs from endogenous development, leading to consequent PSC-CM maturation arrest, are unknown. The advent of single cell RNA-sequencing (scRNA-seq) has offered great opportunities for studying CM maturation at single cell resolution. However, perinatal cardiac scRNA-seq has been limited owing to technical difficulties in the isolation of single CMs. Here, we used our previously developed large particle fluorescence-activated cell sorting approach to generate an scRNA-seq reference of mouse in vivo CM maturation with extensive sampling of perinatal time periods. We subsequently generated isogenic embryonic stem cells and created an in vitro scRNA-seq reference of PSC-CM directed differentiation. Through trajectory reconstruction methods, we identified a perinatal maturation program in endogenous CMs that is poorly recapitulated in vitro. By comparison of our trajectories with previously published human datasets, we identified a network of nine transcription factors (TFs) whose targets are consistently dysregulated in PSC-CMs across species. Notably, we demonstrated that these TFs are only partially activated in common ex vivo approaches to engineer PSC-CM maturation. Our study represents the first direct comparison of CM maturation in vivo and in vitro at the single cell level, and can be leveraged towards improving the clinical viability of PSC-CMs.

Funding Source: This work was supported by grants from NICHD/NIH, AHA, and MSCRF.

Keywords: cardiomyocyte, maturation, single cell RNA-seq

Poster: 504

IDENTIFICATION OF CANCER-RELATED MUTATIONS IN HUMAN PLURIPOTENT STEM CELLS UTILIZING RNA-SEQ ANALYSIS

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Human pluripotent stem cells (hPSCs) are known to acquire genetic aberrations during their in vitro propagation. In addition to recurrent chromosomal aberrations, it has recently been shown that these cells also gain point-mutations in cancer-related genes, predominantly in TP53. To address the critical need for routine quality control of hPSCs, we have recently established a detailed protocol to identify cancer-related point-mutations using data from RNA-sequencing, an assay commonly performed during the growth and differentiation of hPSCs. We describe how to process and align the sequencing data, analyze it, and conservatively interpret the results in order to generate an accurate estimation of mutations in tumor-related genes. The complete pipeline is available as a software tool and is designed to work in high-throughput, providing a simple and precise methodology to inspect the genomic integrity of hPSCs. This protocol enables to perform quality control in high resolution of hPSCs, designated for clinical applications as well as for basic research.

Keywords: Human pluripotent stem cells, Genetic instability, RNA sequencing

Poster: 505

NOVEL IMPRINTED GENES EXEMPLIFY PREDOMINANTLY H3K27ME3-DEPENDENT IMPRINTING IN MOUSE BLASTOCYSTS

Santini, Laura[1], Halbritter, Florian[2], Titz-Teixeira, Fabian[3], Suzuki, Toru[4], Asami, Maki[4], Ma, Xiaoyan[5], Ramesmayer, Julia[1], Lackner, Andreas[6], Pauler, Florian[7], Hippenmeyer, Simon[7], Laue, Ernest[8], Farlik, Matthias[9], Bock, Christoph[9], Beyer, Andreas[3], Perry, Anthony C.F.[4], Leeb, Martin[1]

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Parent-of-origin specific gene expression depends on epigenetic marks (imprints), which are asymmetrically distributed between maternal and paternal mammalian genomes. Genomic imprints correspond to DNA methylation or to recently reported allele specific H3K27me3. However, current maps of the imprinting landscape in early embryos are likely incomplete. We therefore set out to functionally and physically map the parent-of-origin-specific gene expression landscape in mouse preimplantation embryos. Transcriptome profiling of blastocysts from genetically distinct reciprocal crosses revealed 71 novel uniparentally expressed genes (nBiX: novel blastocyst-imprinted expressed genes). We validated candidate genes in independent blastocysts and observed that all tested genes lose their imprinted status upon implantation. To identify the epigenetic mechanisms underlying the monoallelic expression of nBiXs, we performed micro-whole-genome bisulfite sequencing on uniparental blastocysts, uncovering 859 differentially methylated regions (DMRs). Only 16% of nBiXs were associated with a DMR, whereas most were associated with, and/or dependent on, parentally-biased H3K27me3, indicating a major role for Polycomb-mediated imprinting in blastocysts. Many known imprinted genes are located in clusters. We could assign nBiX genes to 5 existing clusters, and further identified 5 new imprinted gene clusters containing exclusively nBiX genes. Collectively, our data suggest a complex program of stage-specific imprinting involving different tiers of regulation.

Keywords: Genomic imprinting, Mouse blastocysts, Early development

Poster: 506

RAPID STEM CELL PROTOCOL ASSESSMENT THROUGH TRANSCRIPTOME-WIDE PROFILING

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Human stem cell protocols that target specific cell lineages can take years to develop and optimize. Development of new protocols, quality control of established protocols across time, or adaptation of established protocols in different hPSC lines and laboratories, all require fast and sensitive validation methods. Here we use a rapid analysis of in vitro RNA-seq data, derived from an array of human stem cell protocols, against an in vivo scRNA-seq reference atlas of the gastrulating mouse embryo. We bioinformatically track the time-dependent maturation of human stem cell protocols targeting multiple germ layers. Further, we directly compare different protocols that target the same cell lineage. This one-step identification of both on- and off-target gene signatures enables rational protocol iteration to either troubleshoot sub-standard stem cell differentiations, or to efficiently develop new stem cell protocols for novel cell types.

Keywords: scRNA-seq, protocol, in vitro

Poster: 507

CODON-OPTIMIZED MINI INTRONIC PLASMIDS ENCODING REPROGRAMMING FACTORS COMBINED WITH ASCORBIC ACID AND VALPROIC ACID SUCCESSFULLY GENERATES INDUCED PLURIPOTENT STEM CELLS FROM HEK-293T CELLS

Sanlioglu, Ahter D.[1], Yilmaz, Ozlem[1], Seker, Gizem[1], Cetin, Busra[1], Akinci, Ersin[1], Altunbas, Hasan A.[2]

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Induced pluripotent stem cells (iPSCs) are widely recognized for their potential of use in various fields of application, including toxicity testing, disease modeling, and gene and cell therapies targeting different diseases. While having close similarities to embryonic stem cells (ESCs), iPSCs are advantageous for surpassing issues such as allogeneic origin and ethical concerns attributed to ESCs. iPSCs are generated via various viral and nonviral means of expression of the reprogramming factors in target cells. However, efficacy and efficiency of the current methodologies need to be detected in different settings and improved with new approaches for more efficient processes. We aimed to generate iPSCs from human embryonic kidney cells (HEK-293T) using codon-optimized mini intronic plasmids (CoMiPs) encoding the OSKM factors (Oct4, Sox2, Klf4, c-Myc), and test the efficacy and efficiency of the process with Ascorbic acid (AA; 50 ug/ml) and Valproic acid (VA; 2 mM), used to improve yield. For this, 4-in-1 CoMiP vectors encoding together the OSKM factors, a short hairpin RNA against p53, and a tdTomato fluorescent reporter were transfected into HEK-293T cells via electroporation at 110V, carried out as a single pulse. Transfection efficacies were evaluated via intensity of red fluorescent signal. DMEM medium was used at initial stages of culture, whereas the reprogramming culture medium TeSR-E7 and maintenance medium TeSR-E8 were used at later stages. Cell morphologies were studied and survival rates were followed-up throughout the process. First iPSC colonies started to emerge at 6-8 days post transfection. We had to adjust (reduce) AA exposure in cultures due to unwanted crystal formation affecting viability. Pluripotency of the emerged colonies were tested via Alkaline phosphatase and Tra-1-81 live staining, both of which were strongly expressed in the emerged colonies. Thus, we generated iPSCs from embryonic HEK-293T cells via use of 4-in-1 CoMiP vectors and small molecules, throughout a relatively short-duration process. This protocol with particular combination, concentrations, and exposure duration and timing of AA and VA use is likely to be effective in establishing iPSCs from a wide range of other cell types, particularly from primary cells, to be tested accordingly.

Funding Source: TUBITAK Project No: 218S617

Keywords: Induced pluripotent stem cells, CoMiP plasmid vectors, Reprogramming

Poster: 508

LNCRNA PLATR21 ENHANCES SOMATIC REPROGRAMMING EFFICIENCY AND MAINTAINS STEM CELL PLURIPOTENCY

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Establishment of the pluripotency-specific chromatin network is crucial in somatic reprogramming. Nevertheless, the epigenetic mechanisms underlying this pluripotent reprogramming remain unclear. To delineate this epigenetic regulatory network, we utilized a “chromatin RNA in situ reverse transcription sequencing” (CRIST-seq) approach to map long noncoding RNAs (lncRNAs) embedded in the 3-dimensional intrachromosomal architecture of stem cell core factor genes. By combining CRIST-seq and RNA-seq, we identified Pluripotency-associated transcript 21 (Platr21) as a pluripotency-associated lncRNA that not only interacts with the Sox2 promoter, but was also activated during the somatic reprogramming to pluripotency. Platr21 expression was associated with the status of stem cell pluripotency. Knockdown of Platr21 caused iPSCs to exit from pluripotency. Platr21 also partially rescued the cell differentiation caused by LIF-withdrawal. In addition, overexpression of Platr21 enhanced reprogramming of mouse embryonic fibroblasts into pluripotent cells. Notably, Platr21-overexpressing embryonic stem cells showed suppressed differentiation into the mesoderm lineage, as demonstrated by low expression of mesoderm markers Brachyury, Twist1, Mixl1, and Hand1. These data suggest that Platr21 may function as a pivotal chromatin epigenetic modulator that coordinates the promoter activity of core stem cell factor genes, highlighting the critical role of pluripotency-associated lncRNAs in the maintenance of stem cell pluripotency and somatic cell reprogramming. This project was supported by NSFC grants 2018YFA0106902, 82050003,

81900701, 31430021, CIRM grant RT2-01942, and Biomedical Research Service of the Department of Veterans Affairs grant BX002905.

Funding Source: This project was supported by NSFC grants 2018YFA0106902, 82050003, 81900701, 31430021, CIRM grant RT2-01942, and Biomedical Research Service of the Department of Veterans Affairs grant BX002905.

Keywords: Long non-coding RNA, stem cell pluripotency, epigenetics

Poster: 509

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF TWO ALTERNATE PROMOTOR REGIONS IN THE SOX17 GENE LOCUS

Trinh, Linh T.[1], Osipovich, Anna B.[2], Sampson, Leesa[3], Wong, Jonathan[4], Wright, Christopher V.[1], Magnuson, Mark A.[2]

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Sox17 is essential for multiple cell lineages during early development. Previous studies have revealed the expression of short and long Sox17 mRNAs in definitive endoderm and in hemogenic vasculature, respectively. We analyzed several published ATAC-seq datasets from endothelial/hematopoietic and definitive endodermal cells and observed two regions of open chromatin within the Sox17 locus that coincide with two evolutionarily conserved regions (CR) that we refer to as CR1 (117 bp) and CR2 (126 bp). To determine whether CR1 and CR2 might function as cell-type specific promoters/regulatory elements for the preferential expression of alternate Sox17 mRNAs in endothelial or endodermal cells, we analyzed their function in mouse embryonic stem cell-derived endoderm or endothelial cells using fusion genes and a reporter assay. CR1 exhibited promoter activity only in the endothelium while CR2 was active in both cell types. To determine the role of CR1 and CR2 in development we also used CRISPR/Cas9 mutagenesis to eliminate these sequences in mice. To our surprise, mice lacking CR1 were viable even though they lack expression of long Sox17 mRNAs, have reduced expression of short mRNA form, and exhibit a modest increase in lympho-vasculogenesis. In contrast, mice lacking CR2 do not live past embryonic day 11.5. Analysis of CR2-null embryos revealed a marked reduction in the short Sox17 mRNA form, the detrimental retention of an intron in the long mRNA forms, and greatly reduced expression of pancreatic, hepatic and biliary markers. Immuno-histological analysis of these embryos also revealed disruption of the cognate primordial tissues. Together, these findings are consistent with there being two cell-type specific promoters of Sox17 with the downstream CR2-associated promoter region being functionally more important than the CR1-associated one. Further investigations will focus on identification of specific transcription factors and distal enhancers that are necessary for the dual lineage-specific expression of Sox17.

Keywords: gene regulation, endoderm, endothelial cell

Poster: 510

TRANSCRIPTIONAL CHANGES DURING MURINE PANCREATIC ENDOCRINE DIFFERENTIATION IN REAL TIME AT SINGLE-CELL LEVEL

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Injury induced loss or dysfunction in hormone secreting pancreatic endocrine cells can be caused by deregulated differentiation or mutations. Such aberrations can result in diseases including diabetes mellitus (DM) and pancreatic neuroendocrine tumours. Both diseases severely impact the patients' quality of life and there is an urgent need for novel and refined therapeutic strategies to treat these diseases. In order to achieve this, knowledge about regulatory factors playing a role in pancreatic endocrine cell lineage specification is required. Following brief expression of the master transcription factor Neurogenin3, endocrine cells are formed during embryonic development. This process has only been characterised for a limited number of time points in mice. The main focus has been on constitutively expressed factors, which are relatively easy to identify, whereas transient and non-coding regulatory factors have remined more elusive. Combining Neurog3Chrono, a bi-fluorescent reporter mouse, and a novel full transcriptome method VASA-seq, we generated a 'real-time'-resolved map of transcriptional changes during pancreatic endocrine development and adult injury. This extensive 'real-time' resource provides more detailed insights into pancreatic endocrine cell formation. Importantly, it allows for identification of novel constitutive and transiently expressed coding as well as non-coding regulatory factors. Identification of regulatory factors driving differentiation of the various types of pancreatic endocrine cells can facilitate ex vivo directed differentiation. It further allows for identification of similarities between neuroendocrine differentiation and cancer, as re-activation of embryonically expressed genes are commonly observed in adult cancer cells. In the long-term, this has the potential to aid the development of cell replacement therapy as well as targeted cancer treatment strategies.

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Keywords: Pancreas, endocrine cells, scRNAseq

Poster: 511

MULTI-ASSIGNMENT CLUSTERING REVEALS SIGNIFICANT PATHWAYS DURING MESODERM AND HEPATOCYTE DIFFERENTIATION

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Clustering represents one of the most fundamental techniques for exploration of high-throughput omics data. The purpose is to partition objects, such as genes or proteins, into a set of clusters based on similarity (e.g., correlation). The final clustering partition is computed such that objects with high similarity end up in the same cluster, and objects with low similarity in different clusters. The assumption behind this analysis is that there exist sets of objects with highly correlated expression profiles and that these may be important for regulation of biological processes and signaling pathways. Thus, clustering analysis can identify hidden structures in data with implications for biological regulation, for instance during stem cell differentiation toward functional cell types. However, commonly applied clustering algorithms like k-means and hierarchical clustering, will identify mutually exclusive clusters. This implies that genes are restricted to being assigned to one cluster only, even though they may have high similarity to several ones. On the other hand, genes with low similarly to all clusters will still be assigned to one. These limitations pose problems for the biological interpretation of clusters, since genes responsible for regulation of a process or pathway may be divided among several clusters, and because low-similarity genes introduce noise. To circumvent these problems, we propose an extended clustering approach called Multi-Assignment Clustering (MAsC). Here we remove the assumption that objects must be assigned into mutually exclusive clusters by setting a threshold on cluster similarity. All objects above the threshold for a given cluster will be assigned to that cluster. This results in a set of partially overlapping clusters, which better approximates the biological context where genes can be involved in regulation of several processes. At the same time, objects with low similarity to all clusters will be dropped from the analysis, thus reducing noise in the clustering partition. When applied to time series data of stem cells undergoing differentiation towards the mesoderm lineage or hepatocytes, we found that MAsC revealed biologically relevant pathways missed by standard k-means clustering. The MAsC algorithm is available on GitLab https://gitlab.com/wolftower/masc.

Keywords: clustering, transcriptomics, time series

Poster: 512

PROTEOMIC PROFILING OF HEPATIC STELLATE CELL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS: NEW TOOLS TO UNDERSTAND LIVER DEVELOPMENT AND FIBROSIS

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Hepatic Stellate Cells (HSCs) are the main fibrogenic cell type in chronic liver disease as they become activated. Understanding the embryonic development of HSCs and their activation is key to develop new therapeutic strategies. By sequential addition of factors, we have generated functional human HSCs from Induced Pluripotent Stem Cells (iPSC-HSC). Here we performed a proteomic analysis to understand how the iPSC-HSC's differentiation mimics the embryonic development and to uncover pathways involved in liver fibrosis. Human iPSC-HSC were obtained following Coll et al. 2018; Vallverdú et al. 2021. Samples were processed every two days for MS-proteome analysis. Four differentiations were analysed and human primary HSCs were used as control. qPCR and FACS analysis were used for validation. The sequential proteomic profiling of the differentiation showed a reduction of pluripotent markers (RIF1, POU5F1, DPPA4) and an increase in HSC markers (LUM, PTN, COL1A1 and MMP2) along differentiation. A Pearson's correlation indicated that differentiation occurred in three stages: undifferentiated phase (day 0 to 4), intermediate foetal stage (day 6 to 8) and final maturation stage (day 10 to 12). Developmental markers of foetal HSC, such as VIM, ALCAM, FBLN1 and DCN were expressed at the foetal stage and were maintained during maturation phase, indicating recapitulation of the embryonic development in vitro. Comparison of iPSC-HSC with primary HSCs revealed that signalling pathways involved in HSC activation such as TGFb, PDGFR, VEGF or autophagy were expressed early at the foetal stage, while most pathways involved in liver fibrosis were not expressed. Moreover, we identified a group of proteins that did not change across differentiation but were highly expressed in primary HSCs. DAVID bioinformatics predicted that RORA could be upstream them, thus regulating HSC differentiation. In vitro experiments with an agonist (SR1078) confirmed that RORA signalling regulates both HSCs differentiation and the quiescent phenotype. The proteomic characterization of iPSC-HSC differentiation uncovered the recapitulation of embryonic development of HSCs and pathways involved in cell activation. This study indicates that iPSC-HSC differentiation is a good model to study HSCs biology and explore mechanisms of liver fibrosis.

Keywords: Proteomic characterization of Hepatic Stellate Cells derived from Induced Pluripotent Stem Cells, Recapitulation of embryonic development of Hepatic Stellate Cells and pathways involved in cell activation, Tools to study liver development and fibrosis

Poster: 513

STEM CELL IDENTITY AND LINEAGE ARCHITECTURE IN RENEWING TISSUES

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What is a (adult) stem cell? The answer to this question is still, after decades of debate, disputed. Commonly, stem cell identity in homeostatic renewing tissues is defined by (1) a cell's potential to self-renew, and thus, to divide without exhaustion, and (2) its potential to generate all cells of a particular cell lineage. Here, we provide a formal framework, based on the topology of admissible cell state trajectories, to show that these two properties are intrinsically intertwined, in so far that they imply each other. This framework defines the relationship between cell molecular `states' and cell `types', and it provides that a self-renewing cell type -- if defined as in our framework -- must reside at the top of any homeostatic renewing lineage hierarchy, and only there. Such lineage architecture arises as a natural consequence of homeostasis, and indeed is the only possible hierarchy of cell types to support homeostasis in renewing tissues. Furthermore, under suitable feedback regulation, for example from the stem cell niche, we show by theoretical arguments that the property of `stemness' is entirely determined

by the cell environment, in accordance with the notion that stem cell identities are contextual and not determined by hard-wired, cell-intrinsic, characteristics.

Funding Source: Medical Research Council: New Investigator Research Grant MR/R026610/1

Keywords: Stem cell identity, Cell state trajectories, Lineage architecture

Poster: 514

EED/H3K27ME3 REGULATES THE TIMING OF MOUSE PRIMORDIAL GERM CELL DIFFERENTIATION

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Primordial germ cells (PGCs) are the founding cells of the entire germline and are required for reproduction. During mammalian PGC development, major epigenetic remodeling occurs to establish an epigenetic landscape for gametogenesis. In both human and mouse PGCs, the global depletion of DNA methylation (5mC) across the genome and the nuclear enrichment of Histone H3 lysine 27 trimethylation (H3K27me3) are among the most well conserved epigenetic changes. While it has been hypothesized that the enrichment of H3K27me3 may play a compensatory repressive role following loss of 5mC, this has yet to be fully explored. To address this, we used the mouse model to create a PGC specific conditional knockout mouse for Embryonic Ectoderm Development (EED), an essential component of polycomb repressive complex 2 which trimethylates H3K27. Through imaging, FACS and RNA sequencing we show that loss of H3K27me3 has no effect on global levels of 5mC and instead that EED/H3K27me3 regulates the timing of PGC differentiation in male and female embryos as well as X chromosome dosage decompensation in male PGCs. Additionally, we identified a subset of intermediate to high GC content gametogenesis promoters in the mouse pluripotent epiblast that exhibit a unique dual H3K27me3 and 5mC repressive signature and provide evidence that EED and DNA methyltransferase 1 (DNMT1) interact in pluripotent mouse cells. Taken together, we propose that EED/H3K27me3 joins DNMT1/5mC as a major regulator of PGC differentiation timing which is wired into the epiblast prior to PGC specification. This work presents a critical advancement in our understanding of mammalian PGC epigenetic regulation with implications upon ways to improve PGC differentiation in vitro from stem cells.

Funding Source: We acknowledge the support of the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Training Program and the Ruth L. Kirschstein National Research Service Award GM007185 for this work.

Keywords: Primordial Germ Cells, H3K27me3, Epigenetic Reprogramming

Poster: 515

HUMAN SKELETAL STEM CELL IDENTITY FROM NEONATES WITH PERINATAL DEATH

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Skeletal stem cells (SSCs) make up the bone stroma and can give rise to cartilage, bone, support for hematopoietic stem cells, bone marrow adipocytes and fibroblasts. Cell differentiation has been mainly described for bone marrow, however, it should also take place in the periosteum and growth plate, where stem cells guarantee tissue maintenance. Hence, SSCs have received attention as potential therapeutic options. However, there is still lack of consensus regarding the surface markers that identify them as SSCs regardless of skeletal element and tissue of isolation. Therefore, the objective of this research is to characterize possible SSC obtained from skeletal elements of newborns with perinatal death through surface marker identification. To this end, surface cell markers were determined by a systematic review in the literature based on a search equation including “skeletal stem cells” OR “Mesenchymal stem cells” and similar terms. Results were from four databases: Medline (462 articles), Scopus (1997), Cochrane (15) and Web of Science (660), for a total of 3,134 research articles out of which 94.06% were excluded based on criteria, such as articles in language other than English (0.15%), animals (2.2%), surface markers in viscerocranial skeleton (0.6%), synovium (0.4%), purchased cells (3.4%), and organs other than skeleton (23%). The 64% excluded articles were related to cell cycle, exosomes, vesicles and transcription factors among others. The most frequently used surface markers were proposed by the ISCT in 2006: CD105 (67.7%), CD90 (61.3%) and CD73 (42.5%). However, other in vitro markers were also identified, such as CD44 (34.4%), CD166 (25.3%), CD29 (22.5%) and Stro-1 (14.5%) in bone marrow (75.8%), cartilage (10.7%) and periosteum (0.5%), the remaining 12.9% did not include site of isolation. In contrast only 3.76% carried out an immunohistochemistry evaluation of marker location. Thus, we will study surface markers on histological sections of skeletal elements, including the head of the femur, body of the vertebra, sternum, and iliac crest, to compare their expression and possible differences depending on tissue location. We propose markers will vary depending on tissue location and skeletal element; suggesting the need to continue demonstrating which cell surface markers are specific for SSCs.

Funding Source: Pontificia Universidad Javeriana VRI 2019 Grant No. 20036 Stromal cells

Keywords: Skeletal stem cells, Surface marker, Newborn

Poster: 517

REPROGRAMMING AND REJUVENATION TRAJECTORIES OF THE DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO INDUCED NEURAL STEM CELLS

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The reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) provided new opportunities for disease modeling. However, during reprogramming the cells are epigenetically rejuvenated, therefore, iPSC-based models are not optimal for studying age-related mechanisms and pathologies. Direct conversion of adult dermal fibroblasts (ADFs) into induced neurons (iNs) circumvents the rejuvenation and allows deciphering mechanisms of cellular ageing. The rejuvenation associated with conversion into proliferating induced neural stem cells (iNSCs) has been poorly investigated. In this study, we aim to study the reprogramming trajectories during the direct conversion of ADFs into iNSCs. Furthermore, we will compare the epigenomic profiles between iNSCs, iNs and iPSC-derived NSCs when converted from ADFs of various age groups. For this purpose, we will employ the CellTagging barcoding methodology to label the cells with 8bp random barcodes at day 0, 7, 13 and 30 during the direct conversion process and collect samples for analyzing the transcriptome and epigenome on a single-cell level. In order to adapt the CellTagging protocol to the direct conversion of human iNSCs, we performed a series of pilot experiments for defining the lentiviral barcoding efficiencies by transducing different cell densities of foreskin fibroblasts, ADFs and iNSCs with a range of viral concentrations between 0.05x - 5x. The infection efficiency was validated by fluorescent microscopy and was quantified by flow cytometry over a period of 10 weeks post-infection. This analysis revealed that foreskin fibroblasts and ADFs were optimally infected with a viral concentration of 2.5x resulting in 98% transduction, while in the case of iNSCs 95% transduction was achieved with an optimal 2x viral concentration. In the next steps of our study, we will perform the sequencing of the barcode library and we will generate a “whitelist” that will be used for the filtering of the CellTags in our final analysis. Finally, we will proceed with the direct conversion experiments, followed by single-cell transcriptomics, high-resolution lineage analysis and DNA methylation analysis. This study will help to exploit reprogramming technologies to gain further insight into neural ageing and regeneration pathways.

Funding Source: FWF SFB F78 Neuro Stem Modulation Marie Curie Actions ARDRE Graduate School

Keywords: Induced neural stem cells, direct conversion, neural ageing and rejuvenation

Poster: 518

LINEAGE REPROGRAMMED HUMAN OLIGODENDROCYTE PROGENITOR CELLS INDUCED BY NGN2 EXPRESSION SHOW EVIDENCE OF NEURONAL PHENOTYPE AND FUNCTION

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Neurons are post-mitotic cells that are not replaced following disease or injury, thus resulting in functional impairment of the central nervous system. Adult neurogenesis does occur, but neuronal replacement is spatially restricted. Nevertheless, the functional integration of these new neurons suggests it may be possible to integrate alternative sources of new neurons into more distributed neuronal circuitry. With the direct reprogramming approach, expression of key developmental transcription factors induce a switch in cell lineage identity that bypasses induction of pluripotency and subsequent differentiation. As a result, direct reprogramming has the potential to target endogenous cells in the central nervous system and engineer their fate to a neuronal identity, providing a novel therapeutic strategy. A number of studies have demonstrated success with this approach using in vitro and in vivo models, but these have been primarily rodent cells. Using cultured primary fetal human oligodendrocyte progenitor cells (hOPCs; gift of Dr. F. Sim, Univ. of Buffalo), we delivered Ngn2-eGFP and eGFP by retrovirus under the control of a constitutive promoter (CAG) or a lineage specific promoter (NG2). Selected by their expression of CD140a to enrich for hOPCs, the predominant cell phenotype upon plating was Olig2+ with a small percentage of GFAP+ cells and no detectable NeuN+ cells. Under growth medium conditions and with optimized viral titer, retroviral constructs containing either CAG-Ngn2-eGFP or NG2-Ngn2-eGFP had about a 50% infection efficiency at 3 dpi which was similar to controls. At two weeks following Ngn2 reprogramming, CAG-Ngn2-eGFP produced 80% of GFP+ cells with a neuronal morphology, while NG2-Ngn2-eGFP resulted in 60% of GFP+ cells appearing neuronal. Neuronal morphology was confirmed by immunostaining for NeuN positivity. Reprogrammed hOPCs were evaluated for neuronal function at 10 and 21 dpi and both CAG and NG2 promoters produced induced neurons with strong inward currents (voltage clamp) and induction of strong spiking activity (current clamp) by 21 dpi. Here we demonstrate that this reprogramming approach successfully induces neurons from human OPCs with mature action potential and excitable membrane properties.

Funding Source: This work supported by NIH NS100514 awarded to DAP, RAM, and GES.

Keywords: glia, induced neurons, repair

Poster: 519

HUMAN PRIMED AND NAÏVE PSCS ARE EQUALLY POTENT IN DIFFERENTIATING INTO BONA FIDE TROPHOBLAST STEM CELLS

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The trophoblast lineage is the first to segregate from other lineages in the developing blastocyst subsequently forming the major part of placental tissues in higher mammals. Recent derivation of human trophoblast stem cells (TSCs) from placental cytotrophoblasts and from blastocyst opens new opportunities for studying the development and function of the human placenta. Until today, it was suggested that applying TSC growth medium on naïve, but not primed, human ESCs converts them to TSCs. The capability of naïve ESCs to convert to TSCs is believed to reflect the higher similarity of naïve ESCs to cells of the early blastocyst. Here, we report on the direct and robust conversion of primed human ESCs and iPSCs into TSCs, either by chemical inhibition of TFGb pathway or by transient overexpression of the YAP gene. The resulting cell lines exhibit self-renewal, are able to differentiate into the main trophoblast lineages, and present RNA and epigenetic profiles that are indistinguishable from the TSC lines previously derived from the placenta or human naïve PSCs. Furthermore, we have found that βCatenin pathway activator CHIR99021, a prevalent component of TSC growth medium, hampers this conversion from primed but not from naïve ESCs, thus explaining previous failure to convert primed ESCs to TSCs.

Keywords: TSC, naive, primed

Poster: 520

TRANSSYNW A SINGLE CELL RNA SEQUENCING BASED WEB APPLICATION TO IMPROVE CELL CONVERSION EXPERIMENTS

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The goal of regenerative medicine is to create functional and faithful cell types that can replace damaged tissues. Although substantial progress has been achieved with novel reprogramming techniques, generating the cells of interest still poses problems. The advent of single cell RNA sequencing (scRNA-seq) opened doors to characterize the heterogeneity within a cell population and allows us to characterize transcription factors (TFs) that drive conversion between subpopulations. However, to successfully convert between the cell types of interest, identity TFs must be able to regulate genes that are silenced and inaccessible for expression in the original cell. Indeed, it has been described that a subset of TFs, denominated as pioneer factors (PFs), possesses the remarkable ability of overcoming such constraints and access genes embedded in closed chromatin, playing a key role in initiating cell conversion mechanisms and improving the efficiency of conversion protocols. Based on this concept, we developed TransSynW, the only computational web application to date that can unbiasedly identify cell conversion TFs for any cell population identified by scRNA-seq data. Based on synergy measurement, TransSynW predicts transcriptional cores that consist of population-specific TFs and non-specifically expressed PFs. Furthermore, it predicts markers for each analyzed population, enabling researchers to assess the performance of their cell conversion experiments. We applied TransSynW to different cell systems and our results well-recapitulated known cell conversion TFs, markers, and made novel predictions in the various systems. In particular, TransSynW identified novel TFs critical for subtype specification of human ventral midbrain dopaminergic neurons (hDA). Using a flexible CRISPR-dCas9 system, we intend to target the predicted TFs and develop a novel protocol that allows the control over hDA subtype generation. Furthermore, by mimicking the cross-repression process that occurs in vivo, we will apply the predicted TFs to simultaneously generate midbrain-specific cell types. Accomplishing efficient cell conversion and providing control over cell type generation will be an unparalleled breakthrough for translational applications, such as cell transplantation and disease modelling.

Funding Source: Fonds National de la Recherche Luxembourg. Grant Number: C17/BM/11662681

Keywords: Cellular therapy, Cell conversion, CRISPR

Poster: 521

A NOVEL FACS BASED CRISPRI SCREENING METHOD TO IDENTIFY STRUCTURAL PERTURBATIONS IN THE NUCLEOLUS

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Human induced pluripotent stem cells (hiPSCs) are unique models for studying normal and pathological conditions. A defective nucleolus is linked to hereditary and neurodegenerative diseases, therefore understanding the basics of nucleolar biology in hiPSCs will help us find the underlying causes of pathologies associated with this structure. We utilized CRISPR interference (CRISPRi) to explore the relationship between nucleolar morphogenesis and structural function in hiPSCs. To visualize the nucleolus we tagged Fibrillarin (FBL-mEGFP) and nucleophosmin (NPM1-mTagRFP-T) to illuminate the dense fibrillar and the granular components of the nucleolus respectively. To systematically investigate genomic perturbations to the nucleolus, we inserted a constitutively expressed CAG-dCas9-KRAB-TagBFP construct into the double-tagged line, creating a platform for CRISPRi screens. We optimized a flow cytometry method that measures how long fluorescent signals take to pass across the laser (wide vs narrow pulse width). We used a pooled library approach targeting approximately 2,300 genes to screen nuclear-focused sgRNAs. We sorted tagged hiPSCs based on their pulse width signal and then used next-generation sequencing to bioinformatically identify candidates. We identified 59 hits that are related to the nucleolus, RNA processing, epigenetic modifiers and transcription regulators. Our validation of the top targets included individually evaluating knockdown capability by genomic analysis, validating pulse-width phenotypes by FACS, and potentially visualizing a spectrum of phenotypes by live imaging. Currently we are continuing validation of the hits that have a distinct nucleolar phenotype in response to knockdown to identify functional genes that relate to nucleolar morphogenesis. The Allen Institute for Cell Science CRISPRi lines AICS-89 and AICS-90 are both publicly available on allencell.org. In summary, our novel FACS-based CRISPRi screen successfully enriched for

morphological changes in pulse width associated with genomic perturbations to the nucleolus, demonstrating the utility of this platform to study genetic determinants of nucleolar structure in the normal and mutant hiPSC lines.

Keywords: CRISPRi, Nucleolus, morphogenesis

12:00 - 13:00 EDT

POSTER SESSION 5

MODELING DEVELOPMENT AND DISEASE

Poster: 530

HUMAN IPSC-DERIVED CARDIOMYOCYTES AS AN IN VITRO MODEL FOR SARS-COV-2 INFECTION

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes cardiac injury in as many as 25% of patients. Despite this, the mechanism by which SARS-CoV-2 damages cardiac cells is poorly understood, and the long-term effects on the patient are largely unknown. Using a simple workflow, induced pluripotent stem cells (iPSC) derived from human patient samples can be differentiated into cardiomyocytes – the major cellular constituent of cardiac muscle – for studying disease mechanisms. These cultures can be dissociated and replated after 10-14 days of differentiation into vessels including 384-well plates, providing a scalable and high-throughput platform for drug toxicity screening and disease modeling. Cardiomyocyte differentiation is confirmed by the expression of cardiac muscle troponin T (TNNT2) and the transcription factor, NK2 homeobox 5 (NKX2-5), and cultures display active beating. iPSC-derived cardiomyocytes express the SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE2), and are a suitable in vitro model for studying response to infection. iPSC-derived cardiomyocytes present a useful solution to understanding SARS-CoV-2 cardiac tropism and have the potential for use in the identification of cardioprotective therapeutics.

Keywords: SARS-CoV-2, Cardiovascular, iPSC-derived

Poster: 531

DEEP LEARNING PREDICTS PATTERNS OF CARDIOTOXICITY IN A HIGH-CONTENT SCREEN USING INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Grafton, Francis[1], Ho, Jaclyn[1], Ranjbarvaziri, Sara[2], Farshidfar, Farshad[3], Budan, Ana[1], Steltzer, Stephanie[1], Maddah, Mahnaz[4], Loewke, Kevin[4], Green, Kristina[1], Patel, Snahel[5], Hoey, Tim[6], Mandegar, Mohammad A.[1]

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Drug-induced cardiotoxicity and hepatotoxicity are major causes of drug attrition. To decrease late-stage drug attrition, pharmaceutical and biotechnology industries need to establish biologically relevant models that use phenotypic screening to predict drug-induced toxicity. In this study, we sought to rapidly detect patterns of cardiotoxicity using high-content image analysis with deep learning and induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs). We screened a library of 1280 bioactive compounds and identified those predicted to have cardiotoxic liabilities using a single-parameter score based on deep learning. Compounds with major predicted cardiotoxicity included DNA intercalators, ion channel blockers, epidermal growth factor receptor, cyclin-dependent kinase, and multi-kinase inhibitors. We also screened a diverse library of molecules with unknown targets and identified chemical frameworks with predicted cardiotoxic liabilities. By using this screening approach during target discovery and lead optimization, we can de-risk early-stage drug discovery. We show that the broad applicability of combining deep learning with iPSC technology is an effective way to interrogate cellular phenotypes and identify drugs that protect against diseased phenotypes and deleterious mutations.

Keywords: iPSC-Cardiomyocyte, Cardiotoxicity, Deep Learning

Poster: 532

MODELING OF A NOVEL FILAMIN-C MUTATION IN HIPSC-DERIVED CARDIOMYOCYTES RESEMBLES RESTRICTIVE CARDIOMYOPATHY PHENOTYPES

Prondzynski, Maksymilian, Gabbin, Beatrice, Liu, Xujie, Pu, William T.

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Pediatric restrictive cardiomyopathy (RCM) continues to have poor outcomes with the lowest pediatric cardiomyopathy survival rates, often leaving heart transplants as the only treatment option. The predominant cause of RCM are genetic mutations in sarcomere and non-sarcomere genes. Up to now pathogenetics and therapeutic targets remain widely unknown. Therefore, we set out to model a novel Filamin-C (FLNC) variant (pGlu2472_Asn2473delAsp), which was identified at the Boston Children's Hospital in a patient diagnosed with RCM. The novel variant was introduced by CRISPR/Cas9 genome editing into healthy control hiPSCs (Ctrl) at the hetero- and homozygous state generating FLNChet and FLNChom iPSCs, respectively. All lines were differentiated to hiPSC-dervied cardiomyocytes (hiPSC-CMs) and modeled in 2D- and 3D-cultures. 2D-cultured mutant hiPSC-CMs revealed increased cell area, multinucleation and myofibrillar disarray after 7 days in vitro (div). These findings were reproduced after 30 div for the FLNChet-CMs. Surprisingly, cell area in FLNChom-CMs decreased after 30 div leaving them significantly smaller than Ctrl-CMs. For 3D modeling engineered heart tissues (EHTs) were cultured for up to 50 div. Force measurements were performed with MUSCLEMOTION and resulted in significantly lower force of contraction in mutant EHTs. Furthermore, arrhythmic beating patterns were particularly evident in FLNChet-EHTs, whereby the FLNChom-EHTs seemed to experience reduced speed of electrical conduction displayed by a wave-like beating pattern. Subsequent immunofluorescent analysis of mutant EHTs revealed isolated islets of hiPSC-CMs surrounded by fragmented nuclei suggesting cell death as the main contributor to the observed functional phenotype.

RT-qPCR analysis resulted in higher expression of stress-induced and proapoptotic genes in mutant EHTs such as BIRC5, SOD2 as well as CASP3, respectively. These findings are in line with associated disease phenotypes in RCM patients often characterized by diminished contractile heart function accompanied by necrosis on cellular level. This study shows that RCM phenotypes can be reproduced in vitro with hiPSC-CMs and may therefore lay the basis for delineating underlying disease mechanisms and testing of novel treatment options in the future.

Keywords: Restrictive Cardiomyopathy, Tissue engineering, Filamin-C

Poster: 533

RARG REGULATES DOXORUBICIN-INDUCED STRESS RESPONSE IN IPSC-DERIVED CARDIOMYOCYTES

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Doxorubicin is a commonly used chemotherapy drug that treats both adult and childhood cancers, but its clinical usefulness is limited by doxorubicin-induced cardiotoxicity (DIC). The incidence of DIC increases up to 65% at cumulative doses of 550 mg/m2, which leads to irreversible heart failure and death. Since some patients suffer from DIC even at low doses, genetic differences may account for some of the inter-individual variability in risk for DIC and several associated genetic variants have been identified. Our preliminary data showed that a missense variant rs2229774 (p. S427L) in retinoic acid receptor gamma (RARG) increases doxorubicin-induced double-strand DNA beaks, reactive oxygen species production and cell death in patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). These direct and causal evidence encourage further investigation into the regulatory roles of RARG and its variant in DIC. Isogenic cell lines were introduced by CRISPR/Cas9 and then differentiated into cardiomyocytes for functional characterizations. RARE-luciferase reporter assay showed RAR regulation activity was activated under doxorubicin treatment and RARG-WT/WT could better respond than WT/S427L. Then we performed RNA-seq in our isogenic cell lines, followed by parallel analyses of a published DIC-related RNA-seq dataset and a RARG targeted gene list that collected from DNase-seq and ChIP-seq databases. Pathway enrichment analysis showed that S427L disrupted RARG targeted signalling pathways activation, leading to decreased DNA repair functions. Our findings reveal the roles of RARG-S427L in transcriptional response to doxorubicin in cardiomyocytes, which provide implications for personalized risk prediction to prevent this adverse drug effect.

Funding Source: This study was funded by the Canadian Institute of Health Research.

Keywords: Doxorubicin-induced cardiotoxicity, Retinoic acid receptor gamma, iPSC-derived cardiomyocytes

Poster: 534

UNDERSTANDING MECHANISMS OF ATRIAL-VENTRICULAR SPECIFICATION AND DIFFERENTIATION DURING EARLY ARDIAC DEVELOPMENT THROUGH SINGLE CELL SEQUENCING

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The molecular mechanisms driving atrial and ventricular fate acquisition in vivo are incompletely understood. We have previous identified that transient expression of Foxa2 during gastrulation specifies a population of cardiac progenitors that give rise to ventricular but not atrial myocytes. In order to understand the transcriptional mechanisms underlying early atrial and ventricular differentiation and specification prior to, and during the morphogenetic events leading to chamber formation, we performed single-cell sequencing (scSeq) on sub-dissected cardiac regions from Foxa2-Cre;mTmG embryos at the cardiac crescent (E8.25), primitive heart tube (E8.75) and late heart tube (E9.25) stages. We performed clustering and differential expression analysis on >10,000 cells at each stage to identify a number of early progenitors and further differentiated cardiomyocytes, as well as the differentially expressed genes and pathways that define them. We also find that Foxa2 lineage-traced cells can be identified transcriptionally by expression of EGFP without the need for cell sorting, allowing for comparison of atrial/ventricular specific progenitors at early stages. We next sought to understand downstream signaling events induced by retinoic acid (RA), which plays a key role in atrial chamber specification and can act as a teratogen during development. We exposed Foxa2;mTmG embryos to various concentrations of RA in utero at E6.5 and found that exogenous RA caused defects in atrial/ventricular chamber size. We then conducted scSeq of RA exposed embryos at equivalent stages profiled previously and used differential expression analysis and lineage trajectory algorithms to understand transcriptional mechanisms driving these changes. In summary, the combination of our Foxa2 lineage traced model with scRNAseq profiling of healthy and RA-injected embryos provides insight into transcriptional mechanisms underlying key events during atrial/ventricular differentiation.

Funding Source: Funding NIH/NHLBI R01HL134956-03

Keywords: cardiac development, single cell sequencing, atrial/ventricular morphogenesis

Poster: 535

MICROPATTERNED HUMAN PLURIPOTENT STEM CELLS ENABLE MODELING OF THE EARLIEST DEVELOPMENTAL STAGES OF CARDIAC VASCULARIZATION

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Elucidation of the earliest stages of simultaneous vascular development with respect to multiple cardiac cell types, along with their spatial distribution, has not been reported. Although model organisms have provided much 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 such early stages of development. Here we show that micropatterned human pluripotent stem cells (hPSCs) enable in vitro modeling of the earliest developmental stages of cardiac vascularization, roughly corresponding to the first three weeks of in vivo human development (Carnegie Stages 9 and 10). Using clues from developmental biology, we are able to create cardiac vascularized and organized tissues (cVOTs) from micropatterning of hPSC colonies by addition of a combination of growth factors that simultaneously give rise to spatiotemporally organized atrial and ventricular cardiomyocytes (CMs), arterial and venous endothelial cells (ECs), multiple smooth muscle cell (SMC) subtypes, multiple fibroblast (FB) subtypes, endocardial cells (ENDOs), epicardial cells (EPIs), progenitor cells (PCs) and various extracellular matrix (ECM) proteins. Using single-cell RNA-sequencing (scRNA-seq), we show similar cellular composition of cVOTs to public scRNA-seq data from a 6.5-week post-conception human embryonic heart (Carnegie Stages 19 and 20). Furthermore, we show cVOTs upregulated NOTCH, BMP, and VEGF canonical pathways known to be essential for vascular development. Using small molecules and known teratogens, we demonstrate inhibition of these pathways and subsequent vascular disruption. Our model provides an in vitro reference for fundamental understanding of early human embryonic cardiovascular development.

Funding Source: NIH-NHLBI K01 Award (HL130608) (OJA), Stanford Cardiovascular Institute (CVI) Seed Grant (OJA), Stanford Maternal and Child Research Institute (MCHRI) Transdisciplinary Initiatives Program (TIP) Award (OJA)

Keywords: cardiovascular development, cardiac vascularization, bioengineered tissues and organoids

Poster: 536

TP53 PROMOTES LINEAGE COMMITMENT OF HUMAN EMBRYONIC STEM CELLS THROUGH CILIOGENESIS AND SONIC HEDGEHOG SIGNALING

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Aneuploidy, defective differentiation, and inactivation of the tumor suppressor TP53 all occur frequently during tumorigenesis. Here, we probe the potential links among these cancer traits by inactivating TP53 in human embryonic stem cells (hESCs). TP53–/– hESCs exhibit increased proliferation rates, mitotic errors, and low-grade structural aneuploidy; produce poorly differentiated immature teratomas in mice; and fail to differentiate into neural progenitor cells (NPC) in vitro. Genome-wide CRISPR screen reveals requirements of ciliogenesis and sonic hedgehog (Shh) pathways for hESC differentiation into NPCs. TP53 deletion causes abnormal ciliogenesis in neural rosettes. In addition to restraining cell proliferation through CDKN1A, TP53 activates the transcription of BBS9, which encodes a ciliogenesis regulator required for proper Shh signaling and NPC formation. This developmentally regulated transcriptional program of TP53 promotes ciliogenesis, restrains Shh signaling, and commits hESCs to neural lineages.

Keywords: neuronal differentiation, TP53, hESCs

Poster: 537

MODELLING SUSCEPTIBILITY TO LIVER STEATOSIS WITH IPSCS OVEREXPRESSING HSD17B13 TRUNCATED SEQUENCE

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Non-alcoholic liver disease (NAFLD) is a multifactorial disease influenced by obesity, metabolic syndrome and genetic background, which is characterized by liver fat accumulation. A genetic variant of HSD17B13 (17-Beta Hydroxysteroid Dehydrogenase 13) which produces a truncated and unfunctional protein, presents a protective role in NAFLD progression. However, the role of this truncated protein in liver cells is unknown. In this study we established an iPSCs line with an inducible overexpression of the HSD17B13 truncated sequence (iPSC-HSD17B13) to evaluate its effect in liver cells modeling NAFLD in vitro. iPSCs with recombinase-mediated cassette exchange (iPSC-RMCE system) were nucleofected with a vector containing an HSD17B13 truncated sequence under a doxycycline-inducible promoter. After positive and negative selection, the iPSCs were characterized by FACS and gene expression. IPSC-HSD17B13 and Wild-type iPSCs lines were differentiated to hepatocytes and hepatic stellate cells (HSCs) adding 0.3mg/mL of doxycycline at day 4 until the end, to induce the expression of the truncated sequence. Hepatocytes derived from iPSCs were stimulated with 400uM of palmitic and oleic acid during 72h to mimic NAFLD steatosis. The HSD17B13 truncated

sequence was correctly integrated in the GFP sequence of the master cell line, as assessed by the loss of GFP expression. Cells overexpressed HSD17B13 truncated sequence after doxycycline exposure validating the efficiency of the system. iPSC-HSD17B13 and Wild-type lines were differentiated to both liver cells resulted in an increased expression of hepatocytes (ALB, CYP3A4 and HNF4a) and HSCs genes (COL1a1, ACTA2 and PDGFRb) and HSD17B13 truncated sequence along the differentiation. Finally, after steatosis induction, hepatocytes overexpressing the HSD17B13 truncated sequence, presented a reduction of hepatocyte damage and inflammatory markers compared to the Wild-type derived cells. This study shows the potential of the iPSC-RMCE system to overexpress target genes in iPSCs and to model diseases. Moreover, our results suggest that HSD17B13 truncated protein may confer a protective effect on steatosis-induced liver injury, thus contributing to prevent NAFLD progression.

Keywords: Overexpression of HSD17B13 truncated sequence in liver cells derived from iPSCs, iPSCs differentiation towards hepatocytes and hepatic stellate cells, Modeling liver steatosis in iPSC derived cells

Poster: 538

HUMAN IPS CELL-DERIVED PODOCYTES ARE SUSCEPTIBLE TO SARS-COV-2 INFECTIONS

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The coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Cells in the respiratory system are the primary target of the virus, but the disease can invade other organ systems. Acute kidney injury has been reported in patients hospitalized with COVID-19 leading to high mortality due to compromised kidney function. Most renal diseases present with massive albuminuria where proteins are lost in the urine. Within the kidney, the podocytes - highly differentiated epithelial cells of the glomerulus, and the endothelial cells constitutes a major part of this filtration barrier by regulating selective filtration across the capillary wall. To help understand renal complications associated with COVID-19, we examined the susceptibility of podocytes to SARS-CoV-2 infections in vitro. Here, we used live SARS-CoV-2 and S-pseudotyped lentiviral particles to study viral entry into the podocytes and how this process affects podocytes and lineage-specific genes. We differentiated podocytes from human iPS cells using our previously established method. These podocytes express cell specific markers at levels comparable to human adult podocytes, and thus more closely resembles specialized cells than those derived from fetal-like organoids. Infection of iPS cell-derived podocytes by SARS-CoV-2 revealed increased uptake and retention of the virus within the cells which was confirmed by qPCR, immunofluorescence and TEM. The presence of the virus in the podocytes altered the expression levels of spike associated genes as well as podocyte-specific genes. Transcriptomic analysis of human iPS cell-derived podocytes revealed a subset of spike receptor proteins that play key roles in SARS-CoV-2 uptake. The expression of these genes in the podocytes compared to Calu-3 and other cell types revealed that the virus might utilize multiple receptors for entry into the podocytes. For instance, ACE2 expression in podocytes is quite low but there is a significant increase in uptake of the virus when compared to Calu-3. Our results suggest that the virus also utilizes the receptor BSG (CD147) for entry into podocytes. Together, these results indicate direct infection of podocytes by SARS-CoV-2, providing an opportunity to understand multiorgan and renal tropism of the pathogenic virus.

Funding Source: Whitehead Scholarship in Biomedical research, Medicine chair's research award, Department of Medicine, Duke University

Keywords: SARS-CoV-2, Renal tropism, Podocytes

Poster: 539

AUTOMATED PLATFORM FOR LARGE COHORTS OF IPSCS DERIVED BETA CELLS

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Directed differentiation of patient-derived pluripotent stem cells represents a powerful tool for studying genotype-phenotype relationships in vitro. 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 the large-scale production of pancreatic beta cells from multiple donors, there are still many technical limitations that need to be overcome, such as line-to-line variation in differentiation efficiency and experimental variation introduced by manual procedures. Moreover, current procedures for large-batch 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®, our automated platform for iPSC derivation, to develop a fully automated, high-throughput platform for the directed differentiation of human pluripotent stem cells into functional pancreatic beta cells. This approach not only addresses the state-of-the-art limitations, but also improves the molecular properties of differentiated tissues; indeed, the automated platform has yielded better marker expression compared to the manual approach throughout the differentiation. Moreover, we closely monitored iPSC differentiation into beta cells via automated procedures for assessing organoid morphology. We have also implemented this differentiation pipeline with automated flow cytometry analysis for the evaluation of developmental marker expression profiling at the stage of Pancreatic Progenitor for the PDX1 and NKX6.1 marker and at the stage of Pancreatic Endocrine Beta Cells for the Insulin and Glucagon marker. Finally, the functional evaluations with Glucose Stimulated Insulin Secretion (GSIS) assays showed consistent fold induction over large cohort of iPSC-derived beta cells. Additional assays including immunohistochemistry and calcium signaling analysis demonstrated a homogenous population of the beta cells forming organoids. Finally, a recent study in a cohort of 12 iPSCs (6 WT and 6 T2D) shows that our automated platform for the derivation of functional pancreatic beta cell organoids is optimized for population-scale disease modeling of diabetes.

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

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CORNING MATRIGEL MATRIX 3D PLATES FOR HIGH-THROUGHPUT PANCREATIC CANCER ORGANOID ASSAYS

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The use of organoids as research tools has become more common due to their ability to better recapitulate disease as compared to more traditional models. Additionally, organoids show great promise in personalized medicine, as biopsies can be used to generate organoids that maintain many functional and genomic characteristics of the donor patient. In order to use organoids as a model, it is essential to maintain proper morphology and polarity. In the body, basement membrane is responsible for helping cells to establish and maintain polarity. This process can be modeled and assayed in vitro by culture of epithelial cells in an extracellular matrix. Corning Matrigel Matrix has been demonstrated to enable polarized epithelial structures to form in vitro and can be used to create and maintain a wide variety of organoid models. To increase the throughput for screening with 3D models, Corning has developed 96- and 384-well microplates pre-dispensed with Matrigel Matrix specifically for 3D applications. The current study highlights the use of pre-coated Matrigel Matrix-3D plates to screen pancreatic cancer organoids. Pancreatic organoids dissociated into single cells and plated onto polymerized Matrigel matrix 3D plates. After 48 hours, organoids were screened against a 272-compound library of cancer drugs, at a final concentration of 10 µM. After 5 days, cell viability was assessed using CellTiter-Glo 3D from Promega. Paclitaxel was used as an internal control for cytotoxic response. Five compounds of interest from the screen were used for dose response testing (Bortezomib, Gemcitabine, Daunorubicin, 5-Fluorouacil, and Oxaliplatin). The results of our experiments showed that Bortezomib, a proteasome inhibitor, was more cytotoxic than Gemcitabine against this pancreatic cancer organoid line. Gemcitabine is often offered as standard of care for pancreatic cancer. This study highlights the value of cancer organoids for a personalized medicine approach.

Keywords: Organoid, Cancer, Screening

Poster: 541

INTERROGATING TRANSCRIPTIONAL REGULATORS AND PATHWAY ANALYSIS IN ISLET DEVELOPMENT AND DIABETES USING SINGLE CELL SEQUENCING

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Pancreatic islets are complex micro-tissues responsible for secreting hormones to regulate blood glucose levels. Because they are highly heterogeneous, studying pancreatic islets require single cell technologies to unmask important endocrine populations, such as the insulin secreting beta cells. Herein, we use modern single cell RNA sequencing technologies and novel bioinformatics tools to profile the transcriptomes of stem cell derived islets (SC islets) and native human islets to study beta cell development and identify key drivers that lead to islet dysfunction in diabetes. SC islets provide a promising source for studying human pancreatic development because of its inherent human origin and developmental routes that are accessible in vitro. We profiled islet differentiations by sequencing a series of stem cell derived samples obtained from multiple stages of islet differentiations from embryonic and induced pluripotent stem cells. These stem cell sources include both healthy and diabetic patients. Herein, we unraveled key genetic regulators driving human islet development, specifically endocrine lineage differentiations and beta cell specification. Differential gene expression and gene set enrichment analysis reveal key pathways such as hippo signaling, and notch signaling to be important for endocrine development. We performed pathway analysis to compare in vitro generated SC islets with native human islets or transplanted mature SC islets and find that the nutrients sensing machinery, stress, and inflammatory gene signatures are linked to beta cell maturity. To further comprehend the effects of diabetes on islet health, we sequenced and analyzed SC islets obtained from diabetic patients and find stress related gene signatures affecting beta cell maturity and functional genes. Altogether, we have compiled a large dataset, highlighting important pathways and genetic regulators in beta cell development and diabetes. Our study provides a powerful resource for identifying targets to improve beta cell differentiations, beta cell health and treatments for type 2 diabetes.

Keywords: Beta Cells, Single-Cell RNA Sequencing, Diabetes

Poster: 542

PLURIPOTENT STEM CELL DERIVED ARTERY AND VEIN ENDOTHELIAL CELLS ILLUMINATE TROPISM OF BIOSAFETY-LEVEL-4 VIRUSES

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The ability to generate human artery and vein endothelial cells (ECs) in vitro from human pluripotent stem cells (hPSCs) would provide a powerful platform to understand their diverse roles in health and disease. However, past efforts to convert hPSCs into ECs were lengthy (e.g., ~6-12 days of differentiation), inefficient (~10%-60% of cells generated being endothelial cells), and typically generated cells that lacked clear artery or vein identity. We devised a strategy to generate human artery and vein ECs with high purity. Using single-cell RNA-seq, we found that our hPSC-derived artery and vein ECs were highly pure (92% and 89% pure for artery and vein induction, respectively). In addition, we showed that our artery and vein ECs showed distinct artery vs. vein transcriptional signatures. By comparing our in vitro-generated hPSC-derived artery and vein cells to published human fetal EC datasets, we showed that our in vitro-generated cells transcriptionally correlated with their in vivo counterparts. Functionally, we showed that our hPSC-derived artery and vein ECs could engraft in mice after subcutaneous transplantation, and in vitro, the cells can sense shear stress, migrate, and uptake low-density lipoproteins. We also showed that artery and vein ECs could be infected by Risk Group 4 viruses in vitro,

which revealed new aspects of the tropism and effects of these fatal viruses. In sum, the ability to generate distinct artery and vein ECs will advance regenerative medicine and the modeling of vascular diseases.

Funding Source: The Thomas and Stacey Siebel Foundation, California Institute of Regenerative Medicine, Maternal & Child Health Research Institute.

Keywords: Endothelial Cells, Biosafety-Level-4 Viruses, Pluripotent Stem Cell Differentiation

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HUMAN PLURIPOTENT STEM CELL-DERIVED VENOUS ANGIOBLASTS MATURE TO FUNCTIONAL LIVER SINUSOIDAL ENDOTHELIAL CELLS UPON INTRAHEPATIC TRANSPLANTATION

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Liver sinusoidal endothelial cells (LSECs) form a highly-specialized microvasculature that plays a critical role in liver function and disease. LSECs function to pass molecules to hepatocytes via fenestrations, scavenge blood-born biomolecules from passing blood, secrete coagulation factor FVIII into the blood, and promote hepatic regeneration. To better understand these roles, we developed a strategy to generate LSECs from human pluripotent stem cells (hPSCs) by first optimizing the specification of arterial and venous endothelial progenitors known as angioblasts through modulation of bFGF, VEGF-A, and Notch signaling and derivative endothelial populations. Induction of an LSEC-like fate by hypoxia, cAMP agonism and TGF-beta inhibition revealed that venous endothelial cells responded more rapidly and robustly than the arterial cells to upregulate LSEC markers and functions in vitro. Upon intrahepatic transplantation in neonatal NSG mice, venous angioblasts engrafted the liver and generated mature, fenestrated LSECs with scavenger functions and molecular profiles of primary human LSECs. When transplanted into the liver of monocrotaline-conditioned adult NSG mice, angioblasts efficiently gave rise to mature LSECs with robust FVIII production. Humanization of the murine liver with 8-12 million hPSC-derived LSECs per mouse provides a proof of concept cell therapy for Hemophilia A, a routinely available source of mature human LSECs, and a tractable system for studying the developmental biology of this key liver cell type.

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Keywords: Liver, Endothelium, FVIII

Poster: 544

LONAFARNIB AND EVEROLIMUS IMPROVE DISEASE SYMPTOMS IN TISSUE ENGINEERED HUMAN BLOOD VESSEL MODEL OF HUTCHINSON-GILFORD PROGERIA SYNDROME

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare, fatal genetic disease caused by accumulation of the toxic protein progerin. Patients exhibit severe progressive atherosclerosis with fatality caused by heart attack or stroke at an average of 14.6 years. With an estimated pool of ~350 HGPS patients worldwide, clinical trials face unique challenges in participant recruitment and sample size, creating a need for accurate preclinical HGPS models. Current 2D cell culture and animal models have several disadvantages in cost, timeline, and biofidelity to the human atherosclerotic disease phenotype. We recently developed a 3D tissue engineered blood vessel (TEBV) HGPS model using patient iPSC-derived smooth muscle (viSMC) and endothelial cells (viEC). HGPS TEBVs are rapidly fabricated in a few hours and replicate HGPS vascular symptoms such as impaired vasoactivity, smooth muscle cell loss, excess ECM deposition, and calcification after 3 weeks of maturation. As the first patient-specific 3D human blood vessel model of HGPS, HGPS TEBVs provide a unique platform for preclinical testing of HGPS therapeutics. There is currently a phase I/II clinical trial testing the combination treatment of Lonafarnib (LF) and Everolimus (Ev). LF is a farnesyltransferase inhibitor that increases weight gain, improves vascular stiffness, and extends survival in HGPS patients. Ev is a mammalian target of rapamycin (mTOR) inhibitor that promotes progerin clearance through autophagy. We tested LF and Ev treatment in HGPS TEBVs to improve vasoactivity, progerin clearance, calcification, and endothelial function. Treating HGPS TEBVs with a combination of 1μM LF and 50nM Ev reduced progerin expression, improved vasoactivity, and increased expression of smooth muscle markers and endothelial markers. TEBV treatment also improved cell density, reduced excess deposition of ECM proteins, alleviated inflammatory adhesion molecule expression, reduced calcification, and decreased apoptosis. HGPS viECs treated with LF had drastically improved nitric oxide production under physiological shear stress (12 dynes/cm2) at comparable levels to healthy viECs. Our study presents a novel in vitro HGPS therapeutic testing platform that demonstrates efficacy of LF and Ev in mitigating HGPS vascular symptoms.

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Keywords: Disease modeling, Tissue engineering, Cardiovascular

Poster: 545

MODELING HUMAN INTESTINAL DEVELOPMENT USING IN VIVO HUMAN INTESTINAL ORGANOIDS

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Tissue engineering of human intestine will allow for the creation of personalized grafts that can circumvent the long-term rejection issues suffered by intestinal transplant patients. Creating this tissue requires understanding the key pathways underlying human intestinal development. While remarkable insight into these pathways has been obtained from animal models, there are critical species-specific differences in intestinal development that require a human facsimile to appreciate. We previously demonstrated that transplanted Human Intestinal Organoids (tHIOs) develop mature intestinal structures, including smooth muscle and a crypt/villus axis. However, it is unknown whether the engraftment and maturation of tHIOs mimics human intestinal development. The aim of this study is to determine whether tHIOs can be used as a model for mechanistic interrogation of human intestinal development. After transplantation, a time course of tHIOs was harvested at two week intervals. Grafts were histologically compared to historical images of developing fetal human intestine to assess similarities. From a histological perspective, anatomical tHIO development mimicked fetal human intestinal development. Immunofluorescence was also performed to further interrogate tHIO maturation over time. While villus maturation spanned all eight weeks of engraftment, OLFM4+ crypts were observed beginning at 4 weeks post transplantation. MKI67 staining revealed proliferation throughout the graft at 2 weeks, but restriction of proliferation to the epithelial stem cell compartment by 8 weeks post transplantation. The muscularis was first observed 2 weeks post transplantation, and, by 6 weeks, had developed into layers. To gain insight about the trajectories of cell types in the developing tHIOs, single nucleus RNA sequencing was performed. Using human fetal single cell datasets, we identified cell type clusters and observed increases in cellular diversity over time. From these observations, tHIOs appear to successfully recapitulate human intestinal development and will serve to close the developmental knowledge gap remaining from animal based studies. The insight gained from this model system will ultimately be used to engineer patient-specific tissue that can be used to treat a variety of intestinal conditions.

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Keywords: intestinal development, Human Intestinal Organoid, modeling

Poster: 546

APILIMOD AND NAFAMOSTAT BLOCK INFECTION OF HUMAN-DERIVED LUNG ORGANOID CELLS BY LENTIVIRUS PSEUDOTYPED WITH SARS-COV-2 SPIKE PROTEIN

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The current pandemic of the disease COVID-19 is caused by widespread transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The main mechanism by which SARS-CoV-2 infects human beings and causes respiratory distress is through infection of type II alveolar in lung tissues. The coronavirus interacts with the ACE2 receptor on the plasma membrane to invade the cell via two endocytotic mechanisms: (1) the early fusion pathway with priming by TMPRSS2 (a membrane serine protease) and (2) the late endosomal pathway without priming by TMPRSS2. To combat the pandemic, there has been a major effort on the part of scientists to repurpose already approved pharmaceutical drugs for potential antiviral properties, such as apilimod, an inhibitor of PIKfyve (a lipid kinase) that blocks endosomal trafficking, and nafamostat, a TMPRSS2 inhibitor. We utilize human induced pluripotent stem cell-derived lung organoids in a 96-well format to test the efficacy of these two drugs in blocking infection by a GFP-lentivirus pseudotyped with the SARS-CoV-2 spike protein concentrations ranging from 3 nM to 10 µM. We report significant reductions in pseudovirus infectivity along higher doses of both apilimod (starting at 0.1 µM, $p < 0.001$) and nafamostat (starting at 0.3 µM, $p < 0.001$). Intriguingly, we also report further reduction in pseudovirus infectivity upon co-treatment with both drugs (various concentrations for apilimod all co-administered with 3 µM nafamostat) and when compared with the negative control DMSO-treated wells (starting at 0.003 µM apilimod, $p < 0.001$) and with 3 µM nafamostat only treated wells (starting at 0.03 µM apilimod, $p < 0.05$). Our experiments provide evidence that pharmacological targeting of both the early fusion pathway and the late endosomal pathway can occlude further infection by SARS-CoV-2. Current and future experiments are underway to test the efficacy and toxicity of these drugs, both alone and in combination, in safely blocking infectivity by SARS-CoV-2 in transgenic human ACE2 mice.

Funding Source: Discovery Stage Research Projects: Quest Award - California Institute for Regenerative Medicine

Keywords: Apilimod, COVID-19, Nafamostat

Poster: 547

DEVELOPMENT OF AN IPSC-BASED TOOLBOX FOR CYSTIC FIBROSIS

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Mutation-specific CFTR modulators are approved for use in a subset of individuals with CF, however hundreds of disease-causing mutations have been described and many patients continue to struggle without targeted therapy. Here, we have reprogrammed iPSCs from individuals with representative class 1-3 CFTR mutations and utilized our airway epithelial and basal cell directed differentiation protocols to generate airway epithelial cell spheroids as well as mucociliary epithelial cultures. We have adapted two previously described functional CFTR assays - forskolin induced swelling (FIS) of 3D spheroids and short-circuit current measurement of a polarized mucociliary epithelial layer. We found that baseline 3D spheroid size varies with the underlying CFTR mutation such that non-CF spheroids are larger than residual function CFTR mutants (G551D), and Phe508del and W1282X spheroids. Analyzing more than 500 spheres per experiment, we tested the FIS response at baseline and in response to known CFTR modulators. G551D spheroids increase to 198% of initial size after VX-770 treatment, compared with vehicle. Treatment of Phe508del spheroids with first-generation CFTR correctors (VX-809 and VX-661) leads to a small FIS effect, significant in only 1/3 Phe508del patient cell lines; however, after treatment with VX-445/661 there is a robust response in 3/3 cell lines (187 +/- 20% size increase across three cell lines; mean +/- SEM). We next generated polarized mucociliary epithelial cells using the same Phe508del lines as above. By first generating NGFR+ airway basal cells, we successfully generated mucociliary cultures in air-liquid interface, which express markers of airway basal cells, secretory cells, and multiciliated cells, with CFTR expression comparable to primary controls. Short-circuit current analyses demonstrate significant improvement in forskolin-generated and CFTR-specific current that varies between cell lines. After treatment with VX-445/661, there is a maximal increase in CFTR-dependent current by 12.9 +/- 0.5 uA/cm^2. In conclusion, we have established an iPSC-based airway epithelial cell platform which detects clinically relevant CFTR modulator effects in two separate functional assays which may be applied to drug discovery and cell-based therapeutic options in the future.

Keywords: cystic fibrosis, drug discovery, airway basal cells

Poster: 548

USE OF PRO-DIFFERENTIATION APPROACH FOR TREATMENT OF COLORECTAL CARCINOMA (CRC)

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Differentiation therapy is a non-conventional therapeutic modality aimed at re-activating endogenous differentiation programs in cancer cells with subsequent tumor cellular maturation and concurrent loss of the tumor phenotype. This approach could be used with chemotherapeutics therapy and/or radiotherapy to promote the differentiation of resistant cancer cells. In this study, we used unbiased computational mathematical tool called Boolean logic to identify invariant genes signatures that drive differentiation of colon stem cells present in the base of the colon crypts and halt the initiation and progression of colorectal adenomas and cancers (CRCs), despite disease heterogeneity. The Boolean logic identified CDX-2 as a differentiation marker for the colon stem cells. Augmentation of CDX-2 can be achieved by administration of specific activators. To generate the proof of mechanisms for our hypothesis, we will treat CPC-APC mice (CDx2-Cre-APC min), the commonly known model for CRC, with the specific activators and assess the effect of these compounds on the CRC progression. In addition, we will isolate the colon stem cells from these mice, grow them as 3D organoids, and then treat them with these activators. We plan to assess the differentiation of 3D organoids +/- activators by microscopy. Also, we will analyze the transcriptome changes in the enteriods by qRT-PCR. Moreover, we will check the differentiation markers in the organoids by confocal microscopy and immunohistochemistry.

Funding Source: This research is being funded by the National Institutes of Health under UG3/NCATS.

Keywords: stem cells, differentiation therapy, colorectal adenomas and cancers

Poster: 549

HUMAN ERYTHROID PROGENITORS ARE DIRECTLY INFECTED BY SARS-COV-2: IMPLICATIONS FOR EMERGING ERYTHROPOIESIS IN SEVERE COVID19 PATIENTS

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We document here that intensive care COVID19 patients suffer a profound decline in hemoglobin levels but show an increase of circulating nucleated red cells, suggesting that SARS-CoV-2 infection either directly or indirectly induces stress erythropoiesis. We show that ACE2 expression peaks during erythropoiesis and renders erythroid progenitors vulnerable to infection by SARS-CoV-2. Early erythroid progenitors, defined as CD34-CD117+CD71+CD235a-, show the highest levels of ACE2 and constitute the primary target cell to be infected during erythropoiesis. SARS-CoV-2 causes the expansion of colony formation by erythroid progenitors and can be detected in these cells after two weeks of the initial infection. The results we show here constitute the first evidence of direct infection of specific erythroid progenitors (ERP) by SARS-CoV-2. The work we present here might help understand the emergent erythropoiesis and aberrant presence of erythroid progenitors in the peripheral blood of severe COVID-19 patients. These recent evidences indicate that the increase of erythroid progenitors in circulation constitute a hallmark of both severity and fatality in COVID-19 patients. In this context, our observations that the virus can be detected after 14 days in the ERP-S2 without impairing their viability, rather causing an increase of colonies, suggest that the expansion of circulating erythroid cells that have been reported in severe patients may be due to direct infection of upstream CD71+CD235a- progenitors. Considering their high proliferative capacity, the infection of these progenitors by SARS-CoV-2 may have a major detrimental impact not only in erythropoiesis, but also in the spread of the virus through millions of circulating infected cells. Also, this presents a scenario in which infected erythroid progenitors may cause local

inflammation in the bone marrow, which could cause a drastic disruption of hematopoiesis and the production of immune cells. In contrast to the observations in erythroid progenitors, we report that bone marrow HSPCs (CD34+CD38-) do not express ACE2 and TMPRSS2 at the RNA or protein level. Consequently, we show that HSPCs are not infected by SARS-CoV-2.

Keywords: Erythroid progenitors, SARS-CoV-2, Hemoglobin

Poster: 550

CAN YOU DO THAT HERE? A SURVEY OF NATIONAL AND US STATE POLICIES FOR HUMAN EMBRYO AND EMBRYOID RESEARCH

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Research using human embryos and embryoids has expanded in recent years due to technological advances. However, some of this research is limited by national policies which can restrict work in many, but not all, countries. For example, in the US, there are federal restrictions on funding research that could create or destroy a human embryo, but no national policy regulates non-federally funded work. As a result, human embryo research is funded only by state governments and private institutions, with the policies governing this work shaped predominately at the state level. Human embryoid research, in contrast, is too new to have direct legislation, although existing national and state laws can still limit this work. This presentation will survey national laws that impact human embryo and embryoid research. It will also review the landscape of US state laws that guide human embryo and embryoid research, highlighting gaps existing or uncertainties at both the federal and state levels. While the majority of research-intensive countries have policies, not all are permissive. In addition, the US is not alone in having no national policy to regulate all of human embryo research. US state laws vary tremendously from permissive to bans on all research, and many of the restrictive policies are influenced by abortion politics. Further, while many state and national laws address or impact human embryo research, none of the laws explicitly deal with human embryoid research and therefore their effect on this area of research is unclear in many cases and in need of further guidance from state or federal authorities. As scientists begin to develop the technological capacity to grow human embryos in vitro for longer periods and develop more sophisticated embryoids that mimic later developmental stages, they will have to contend with the amalgamation of often unclear state and national laws and regulations that impacts their work.

Keywords: public policy, human embryos, human embryoids

Poster: 551

TRANSIENT EXPRESSION OF HOXA9 IN A PLURIPOTENT STEM CELL DERIVED MODEL OF HEMATOPOIESIS

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HOXA genes have key roles in regulating hematopoiesis. In mice, Hoxa genes are expressed in hematopoietic stem cells (HSCs) and govern commitment to erythroid, myeloid and lymphoid lineages. Hoxa9 is the most highly expressed Hoxa gene in mouse HSCs, regulating self-renewal and engraftment in vivo. Human pluripotent stem cell (hPSC)-derived hematopoietic stem/progenitor cells (HSPCs) lack HOXA expression and in vivo repopulation potential, in contrast to human fetal liver and cord blood-derived HSPCs. These discrepancies suggest HOXA may be a key regulator of definitive hematopoiesis during in vitro differentiation. Divergence of primitive and definitive fates is proposed to occur during mesoderm specification. Modulation of WNT and ACTIVIN pathways in mesoderm upregulates HOXA, generating cells reminiscent of intra-embryonic hematopoiesis. The mechanism via which HOXA genes may regulate definitive specification remains largely unknown. In hPSC models of definitive hematopoiesis, HOXA9 expression has been identified at key lineage commitment stages, namely mesoderm; arterial hemogenic endothelium; and HSPC. Given its prevalence, HOXA9 may represent the central gene in definitive hematopoiesis regulation. Here, we investigate the role of HOXA9 in definitive lineage commitment in hPSC-based hematopoietic differentiation. A doxycycline-inducible HOXA9-mScarlet expression system targeted to the AAVS1 locus was generated in hPSCs and verified by QPCR, immunostaining and western blot analysis. Transient HOXA9 expression during mesoderm patterning was tightly regulated by doxycycline and monitored in real-time via mScarlet fluorescent microscopy. Preliminary data demonstrates specification of KDR+CD235a- hemogenic mesoderm was sensitive to HOXA9 dosage and duration, resulting in multipotent CD34+CD43+CD90+CD38- HSPCs. HOXA9 overexpression during mesoderm promoted gradual upregulation of endogenous HOXA9 as differentiation progressed, obtaining highest level in HSPCs. Thus, HOXA9 represents a potential master regulator of definitive hematopoiesis. Subsequent studies will utilize single cell RNA-seq to identify downstream targets and processes associated with HOXA9 expression in KDR+CD235a- mesoderm cells.

Funding Source: Joan Browne Legacy Scholarship

Keywords: HOXA9, Definitive hematopoiesis, Inducible expression system

Poster: 552

LEVERAGING SINGLE CELL RNA-SEQUENCING TO STUDY ROLE OF PERSISTENT ARYL HYDROCARBON RECEPTOR (AHR) SIGNALING IN HUMAN HEMATOPOIESIS FROM HEMATOPOIETIC STEM CELLS

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Persistent AHR signaling modulates multiple branches of human hematopoiesis by mechanisms that are not well understood. Single cell transcriptomics was employed to study the effect of AHR activation by 1 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early stages (14 days of culture) of hematopoietic development. An in vitro culture system was used that favors B lymphocyte development from human cord blood derived CD34+ hematopoietic stem and progenitor cells (HSPCs) but also allows development of precursors belonging to other hematopoietic lineages. The aim was to determine how persistent AHR signaling modulates gene expression in differentiating HSPCs and regulates development of different hematopoietic cells. Cell clusters were generated based on gene expression using machine learning algorithms and annotated based on

characteristic hematopoietic markers. Cells belonging to plasmacytoid dendritic cell (pDC) and megakaryocyte-erythroid (MEk) lineages were drastically reduced with reductions in pDC and MEk progenitors in TCDD-treated group being 21% and 32% of that of vehicle (0.02% DMSO) on Day 14. However, monocyte and neutrophil progenitor populations showed an increase, suggesting that persistent AHR activation skews hematopoiesis towards particular myeloid lineages. Flow cytometric analysis confirmed reduction in common lymphoid progenitors, early and Pro-B cells as well as a decrease in pDCs (15% of vehicle) with TCDD treatment at Day 14. Differential gene expression analysis revealed genes critical for pDC development (SPIB, TCF4, BCL11A) and hemoglobin-associated genes were downregulated (log2 fold change difference > 0.25, p value < 0.01). Functional enrichment analysis of differentially expressed genes showed an increase in inflammatory pathway response in most cell types. Overall, this study reveals how persistent AHR signaling modulates distinct gene expression programs in differentiating HSPCs and regulates development of different hematopoietic lineages.

Funding Source: The study is supported by NIH P42 ES004911.

Keywords: AHR, Hematopoiesis, scRNA-Seq

Poster: 553

DBR1 IS A HUMAN MICROGLIA-INTRINSIC VIRAL RESTRICTION FACTOR

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Primary infection with herpes simplex virus type 1 (HSV-1) can precipitate herpes simplex encephalitis (HSE), the most common sporadic form of viral encephalitis in humans. Inborn errors of cell-intrinsic immunity in the central nervous system (CNS) can account for HSE, with defective TLR3 (Toll like receptor 3)- and DBR1 (RNA lariat debranching enzyme)-mediated immunity underlying forebrain and brainstem HSE, respectively. Both the cellular and molecular mechanisms underlying brainstem encephalitis following DBR1 deficiency have so far been elusive. We report that DBR1 functions as an essential antiviral host factor in human microglia, as DBR1-mutated patient iPSC-derived and as CRISPR/Cas9-engineered hPSC isogenic DBR1-mutant microglia are highly susceptible to HSV-1 and IAV (Influenza A virus) infection, as opposed to their WT counterparts. Our mechanistic studies in hPSC-derived microglia show that, in contrast to hPSC-cortical neurons, TLR3-IFN signaling is redundant for microglia cell-intrinsic antiviral immunity, suggesting that DBR1 deficiency impairs intrinsic immunity by a distinctive mechanism independent of known anti-viral signaling pathways. Exogenous IFN-alpha only partially rescued DBR1-mutated microglia resistant to viral infection. Following viral infection, we observe an abnormal type I and III IFN response in DBR1-mutated microglia. Transplantation of DBR1-mutant microglia into WT cerebral organoids is sufficient to induce increased viral replication following HSV-1 infection. At basal levels, DBR1 deficient microglia secrete increased levels of pro-inflammatory cytokines and exhibit elevated levels of phagocytosis. Based on our mechanistic studies, we propose that DBR1 is an essential microglia-intrinsic viral restriction factor and that DBR1 deficiency leads to an aberrantly activated microglial state.

Funding Source: O.H. was supported by a training award from the NYSTEM grant #C32559GG, a NARSAD Young Investigator Award, and the Center for Stem Cell Biology at MSK.

Keywords: Encephalitis, Microglia, HSV-1

Poster: 554

EFFECT OF INTERLEUKIN 1 BETA STIMULATION ON INFLAMMATORY SIGNALLING PATHWAYS IN EQUINE ADULT AND EMBRYONIC STEM CELL-DERIVED TENOCYTES

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Tendon injuries are common in racing thoroughbreds and exercising humans. The deposition of biomechanically inferior scar tissue leads to high re-injury rates, with the proinflammatory cytokine interleukin 1 beta (IL-1β) suggested to mediate tendon degeneration in vivo. Exposure to IL-1β in vitro has negative effects on adult tenocytes in 2D and 3D culture, but embryonic stem cell (ESC)-tenocytes are protected. Differential activation of the NF-κB pathway is suggested to mediate these effects, although it is unknown if other pathways are differentially activated by IL-1β. Such findings could elucidate novel pharmaceuticals to protect endogenous tendon cells from inflammation. In 2D, adult tenocytes were exposed to IL-1β in a time-and dose-response manner, with subsequent immunocytochemical analysis of NF-κB and JNK cytosol-nuclear translocation. Furthermore, employing the first RNA-Sequencing analysis of equine tenocytes following IL-1β exposure, we aim to identify changes in global gene expression profiles between adult and ESC-tenocytes cultured in 3D collagen gels. Tenocyte function will be assessed via daily gel contraction analysis with qPCR validation of selected genes at day 14. Immunocytochemistry revealed that IL-1β at 1 nM enhanced NF-κB cytosol-nuclear shuttling in adult tenocytes after 20 minutes, with peak translocation at 60 minutes. IL-1β had no effect on JNK nuclear translocation at any timepoint. IL-1β reduced 3D gel contraction and increased the expression of matrix metalloproteinases at day 14. Finally, several tendon-associated genes were attenuated by IL-1β. Our findings show that IL-1β enhances NF-κB, but not JNK, cytosol-nuclear translocation in equine adult tenocytes. Furthermore, IL-1β inhibits equine tenocyte function and modulates the expression of tendon associated and extracellular matrix remodelling genes after 14 days in 3D culture, suggesting negative consequences for tendon regeneration in vivo. While additional inflammatory pathways and cytokines may be involved in facilitating the negative effects of inflammation on equine tendon degeneration, pharmacological modulation of the IL-1β-NF-κB pathway during the initial inflammatory phase post-tendon injury might be a promising strategy to improve long-term tendon healing in horses.

Funding Source: Petplan Charitble Trust

Keywords: Musculoskeletal, Inflammation, Tendon

Poster: 555

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

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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 forebrain-type organoids derived from patients and genome edited human induced pluripotent stem cells (iPSC) to address pathophysiological changes associated with a subcortical band heterotopia (SBH) caused by mutations in the EML1-gene. This SBH is characterized by a megalencephaly, a ribbon-like heterotopia and an overlying polymicrogyria-like cortex in the region of the outer SVZ, resembling a second inner cortex. We found that EML1-patient and KO-derived organoids reflect a SBH-like phenotype with ectopic proliferating radial glial cells (RGC) accumulating at the basal side of the cortical structures and neurons organized into two bands, above and below the ectopic cells. Single-cell RNA sequencing and immunohistochemistry reveal an upregulation of a set of extracellular matrix (ECM) components and genes involved in neuronal fate acquisition in the ectopic cell population. To further investigate the origin of the ectopically located cells we assessed apical RGC cell delamination. Control-EML1-hybrid organoids point towards a cell-autonomous delamination defect in EML1-deficient RGCs. We found significant changes in the cleavage angle of EML1-deficient cells shifting the mode of cell division towards oblique and/or horizontal angels, reduced length as well as perturbed structures of their primary cilia and defective regulation of the mechanically sensitive yes-associated protein (YAP). Thus, our data hint at the possibility that perturbed delamination of EML1-deficient RGCs from the VZ might be the primary cause of the heterotopia phenotype and suggest that organoid-based systems serve as promising models to study early human cortical development and associated disorders.

Keywords: Cerebral Organoids, Subcortical Band Heterotopia, EML1

Poster: 556

DISEASE SIGNATURES OF SPINAL MUSCULAR ATROPHY IN CNS ORGANOIDS REVERTED BY PEPTIDE-ANTISENSE OLIGONUCLEOTIDES

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Spinal muscular atrophy (SMA) is a neuromuscular disorder caused by recessive mutations in SMN1 and represents the leading genetic cause of childhood death worldwide1,2. Recent advances in the treatment of SMA have successfully modified its natural history3, but there is still great heterogeneity in clinical outcome and therapeutic window. Understanding the role of SMN in early stages of pathogenesis within the spinal cord and, more broadly, in the central nervous system (CNS), is pivotal to optimizing therapeutic approaches. Here, we generated a novel 3D organoid in vitro model of human spinal cord (SCOs) to study SMA pathology from patient-derived induced pluripotent stem cells (iPSCs). As highlighted by single cell transcriptomics, cellular heterogeneity and key events of spinal cord development were reproducibly recapitulated in SCOs. Morphological and histological analyses detected early neuronal differentiation defects in SMA SCOs, in addition to established pathological hallmarks. High-density multi-electrode array (MEA) recording revealed distinct basal activity and hyper excitability in SMA SCOs. Notably, disease-specific cellular and functional features were restored by treatment with an optimized antisense oligonucleotide (ASO) targeting SMN2. We extended our studies to SMA cerebral organoids and detected corresponding alterations in activity compared to controls, supporting the early-onset multisystemic nature of the disease.

Funding Source: Italian Ministry of Health grant RF-2018-12366357

Keywords: Central Nervous system organoids, Spinal Muscular Atrophy, Therapy

Poster: 557

STEM CELL MODELING OF GTPCH DEFICIENCY IN 2D AND 3D REVEALS CHANGES IN ANTIOXIDANT CAPACITY BUT NOT DIFFERENTIATION CAPACITY COMPARED TO HEALTHY CONTROLS

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Guanosine triphosphate (GTP) cyclohydrolase I (GTPCH, encoded by GCH1; OMIM 600225) catalyses the conversion of GTP to dihydroneopterin triphosphate, the initiating step in the biosynthesis of tetrahydrobiopterin (BH4). This is the rate-limiting enzyme of BH4 generation. BH4 serves as essential cofactor for aromatic amino acid hydroxylases (tyrosine hydroxylase (TH), phenylalanine hydroxylase (PAH), tryptophan hydroxylase (TPH)), nitric oxide synthases (NOS1-3), and alkylglycerol monooxygenase (AGMO). Congenital defects of GCH lead to severe central deficiency of BH4, and subsequently to reduced availability of dopamine, serotonin, epinephrine, and norepinephrine. Affected patients present with neurological symptoms reaching from focal dopa-responsive dystonia to severe infantile Parkinsonism. Low levels of BH4 have also been associated with uncoupling of NO-synthesis resulting in large amounts of reactive oxygen species (ROS). Here we report the generation of GTPCH-deficient (GCHD) patient-specific iPSCs, their characterization and differentiation into neural progenitor cells (NPCs) and dopaminergic neurons. RNA-Sequencing data and metabolite measurements, explored using integrative bioinformatic analysis, revealed reduced expression of genes involved in neuron differentiation and regionalization (forebrain, cortex), but upregulation of gene sets involved in glial differentiation. Nevertheless, differentiation in 3D to cerebral organoids showed no obvious signs of enhanced glial differentiation until day 90 of differentiation but slight changes in the timing of differentiation. Furthermore, unfolded protein response genes and genes involved in reactive oxygen species pathways were also significantly upregulated compared to wildtype control cells in our RNA-Seq data set. As expected, GCHD NPCs are more susceptible to oxidative stress when challenged with the redox-cycling compound menadione or the peroxynitrite generator SIN-1 compared to healthy wild-type NPCs. ROS and peroxynitrite scavengers, including α-tocopherol and ebselen, can only partially rescue GCHD NPCs. Hence, BH4-deficient cells seem to be more prone to oxidative stress which may have implications for the understanding of the pathophysiology and the therapeutic regimen.

Funding Source: Dietmar Hopp Stiftung (https://dietmar-hopp-stiftung.de/)

Keywords: Rare neurometabolic disease, Cerebral organoids, Oxidative Stress

Poster: 558

GENOME-ENGINEERED HUMAN STEM CELLS TO STUDY THE PATHOPHYSIOLOGY OF ASD-RELATED VGCC MUTATIONS

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Voltage-gated calcium channels (VGCCs) are key modulators of early neural development and ensure functionality of the adult human brain. Mutated VGCCs contribute to a range of neurological disorders, including Autism-Spectrum-Disorders (ASD). The aim of this study is to decipher the role of two de novo mutations (V401L and A749G) in the Cav1.3 VGCC subunit encoding gene CACNA1D, which have been reported in patients with a neurodevelopmental disease spectrum including ASD. Our previous electrophysiological studies in tSA cells indicate that these mutations affect the gating properties of Cav1.3, resulting a gain-of-channel-function, by lowering the voltage-dependencies of channel activation and inactivation. However, no data is available on how these mutations affect disease-relevant human neurons. Here we report the CRISPR/Cas9-mediated genome editing and validation of human neural stem cells (NSCs) to introduce the mutations of interest. In detail, we used the optimized Cas9 VRER variant in combination with a specifically designed single-stranded DNA repair template followed by RLFP-based screening, to facilitate highly efficient editing in NSCs with minimal off-target effects. Comparison of different plasmid purification approaches and transfection reagents was imperative to achieve efficient (>40% transfected cells) delivery of the CRISPR components into the NSCs. For functional studies we subjected NSCs to in vitro differentiation protocols to obtain human neurons, showing spontaneous activity during patch-clamp recordings after 4.5 weeks of differentiation. A prerequisite for this study is the analysis of gene expression patterns, not only of CACNA1D, but also of other VGCC subunit genes, and their splice variants, throughout the stages of differentiation. To this end, existing RNASeq data of iPSC-based cortical differentiation has been analyzed to map VGCC subunit expression over time, which will be complemented by qPCR data from future differentiations. Through ICC stainings, electrophysiological recordings and calcium imaging we will investigate how the mutations affect neurodevelopment and neuronal function, which will broaden our knowledge regarding the role of VGCC subunits in the human brain and will overall deepen our understanding of the pathophysiology underlying ASD.

Funding Source: Austrian Science Fund (FWF P27809), EURAC, AGE_REG

Keywords: voltage-gated calcium channels, Autism-Spectrum-Disorder, iPSC-based disease modelling

Poster: 559

A MODULAR BRAIN-ON-CHIP FOR MODELLING EPILEPTIC SEIZURES WITH FUNCTIONALLY CONNECTED HUMAN NEURONAL NETWORKS

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Epilepsy is a complex neurological disorder affecting over 50 million people worldwide. This disease of neuronal networks consists of seizures that affect few networks of the brain (focal seizures) or the entire brain circuitry (generalized seizures). For many epilepsy forms, there is no permanent cure and with the current antiepileptic drugs available, about a third of the patients are refractory to the treatment and are left with persisting symptoms, which raises the need for improved human specific pre-clinical disease models. The use of human pluripotent stem cells (hPSCs) are advantageous for modelling epilepsy, but alone they cannot model interconnected neuronal

networks. Our approach is therefore to create a novel brain-on-chip platform that can monitor the event of seizure like activity in hPSC-derived cortical neuronal networks. The Modular platform for epilepsy modelling in vitro (MEMO) was thus developed. Cortical neuronal cultures are compartmentalized in a custom-made microfluidic device and functionally interconnected through the microtunnels. The event of electrophysiological cell activity is monitored on a custom-made microelectrode array (MEA), assembled beneath the microfluidic device. We show that in MEMO neuronal networks develop well and are able to be cultured for up to 98 days. To create a phenotype that models seizure or epileptic-like behaviour, cells were treated with a seizure inducing substance, kainic acid, and counteracted with an anti-seizure inducing substance, phenytoin. The kainic acid treated networks increased bursting activity and remained localized to that region and similarly with phenytoin. MEMO is thus a suitable platform for modelling focal seizure behaviour in vitro and pharmacological responses.

Funding Source: Academy of Finland and the Finnish epilepsy foundation

Keywords: -Epilepsy -In vitro disease modelling, -Human stem cell derived neurons -Functional circuitry, -Microfluidic device -Microelectrode arrays

Poster: 560

INDUCED NEURONS TO EVALUATE PSYCHIATRIC RISK FOR ALZHEIMER'S DISEASE DEVELOPMENT AND PROGRESSION

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The number of elderly humans is growing, and incidences of age-dependent neurodegenerative diseases such as Alzheimer's disease (AD) are rising. This is alarming, because the development of disease-modifying treatments is hampered by incomplete understanding of age-dependent pathogenic mechanisms of AD, plus the unavailability of early diagnostics to design clinical trials for preventative therapy. Thus, identification of early disease signatures and pathways will be essential to develop future treatment. The impact of late-life psychiatric diseases on the progression of a healthy cognitive state to a neurodegenerative one is coming into the limelight of AD research. Recent studies demonstrated compelling evidence that patients suffering from severe psychiatric illnesses show a significant acceleration of various aging signatures, and are of higher risk to quickly convert from mild cognitive impaired (MCI) into AD. These observations prompt the question to what extent late-life psychiatric diseases may contribute to the initiation and progression of neurodegeneration through the acceleration of biological aging. To illuminate the interface between psychiatric and neurodegenerative pathways in neurons, we generated and characterized age-equivalent induced neurons (iNs) from a neurodegeneration cohort. This cohort ranges from cognitively healthy, to MCI, and further to cognitively impaired AD patients of diverse clinical stages. MCI patients show varying degrees of psychiatric illness, and we further include well-defined cognitively normal psychiatric patients. Thus far, we have generated iNs from over 70 subjects to reconstruct the neurodegeneration trajectory. To evaluate its relationship with psychiatric disease signatures, we performed paralleled genome-wide transcriptomic and epigenomic analyses. Transcriptome analysis revealed distinct signatures in several subgroups of the cohort, and epigenetic aging analysis indicates accelerated aging in iNs associated with psychiatric risk. Next steps will include multi-omics analyses, integrative meta-analyses, and functional assays to validate and better understand the underlying cellular and molecular mechanisms relevant to psychiatric risk factors for neurodegeneration.

Keywords: Alzheimer's Disease, Mild Cognitive Impairment, Psychiatric disease

Poster: 561

THREE-DIMENSIONAL GELMA HYDROGEL COMBINED WITH ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS FACILITATE REGENERATION FOLLOWING SPINAL CORD INJURY

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Spinal cord injury (SCI) is a devasting health issue that occurs with high incidence in young adults. Current therapeutic strategies for SCI includes surgical decompression and physiotherapy. Given that, there is no gold standard treatment for spinal cord injury, new therapeutic strategies with neuro-regenerative and neuro-protective prosperities are required. Adipose derived mesenchymal stromal cells (ASCs) have been promising for neuronal regeneration due to their regenerative capacity and multipotency. Three-dimensional (3D) soft gelatin methacryloyl (GelMA) (5%) hydrogel provides superior mechanical properties and functional characteristics for cell proliferation, migration, and differentiation. In this study, we proposed a functional scaffold developed by loading ASCs into 3D GelMA hydrogel for neuronal regeneration in SCI. ASCs, photoencapsulated in the 3D GelMA hydrogel survived and demonstrated good proliferation in vitro and in vivo for 7 days. The 3D GelMA hydrogel showed in vivo compatibility and promoted angiogenesis when administered to 7E chick embryos. When ASCs-laden hydrogels were implanted into the hemisection site of the rat spinal cord, they filled the destroyed gap and merged in good form with the surrounding tissue, and no cavities were observed between the scaffold and the surrounding tissue. Organized regenerated tissue was observed., We conclude that transplantation of scaffolds combined with ASCs enhanced the regeneration of spinal cord, propose loading of the GelMA scaffold with ASCs as a promising therapeutic strategy to trigger functional regeneration of the spinal cord.

Funding Source: This work is supported by The American University in Cairo (AUC) research support grant and by grant #5300, funded by the Egyptian Science and Technology Development Fund (STDF).

Keywords: 3D GelMA hydrogel, Spinal cord injury, Adipose derived mesenchymal stromal cells

Poster: 562

LITHIUM TREATMENT INDUCES INCREASED EXPRESSION OF TET3 AND GAD2 IN IRRADIATED HUMAN NEURAL STEM AND PROGENITOR CELLS

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Cranial radiation is a common therapeutic approach for childhood brain cancers, such as medulloblastoma, but has been associated with late-effects, such as decline in cognition, mood, and social competence. Hence, treatments harnessing neurogenesis are currently of great relevance in this context. Lithium, a well-known mood stabilizer, has both neuroprotective, pro-neurogenic, and antitumor effects and has been found to reverse radiation-induced damage in mice. We recently linked these effects to promoter demethylation and increased levels of the tubulin polymerisation promoting protein (TPPP) – associated with myelination - and glutamate decarboxylase 2 (GAD2) - associated with neuronal signaling and differentiation - in rodent neural progenitors (Zanni et al., Mol. Psychiatry, 2021). Whether this lithium-induced up-regulation and demethylation also occurs in human cells is unknown, and the demethylation mechanism is unclear. Here, we established a protocol for irradiation and lithium treatment in human induced pluripotent stem cell (iPSC) - derived neuroepithelial stem and progenitor cells (hNSPCs). The gene expression profiles of TPPP and GAD2 were studied in hNSPCs irradiated with a dose of 4 GY and treated with 3mM LiCl for 3 hours, in order to investigate putative pro-proliferative effects of lithium and the expression of Ten-Eleven-Translocation (TET) proteins, associated with reduced cytosine methylation, was investigated for their potential role in the demethylation process. We observed almost ten-fold increase of GAD2 expression in irradiated and LiCl treated hNSPCs compared to the control after 48 hours ($p=0.03$, $n=9$), a trend for a two-fold increase of TPPP expression and a two-fold increase of TET3 expression in the same group compared to the control ($p=0.007$, $n=9$). Our results suggest that TET3 may be involved in the lithium-induced effects on gene expression specifically after irradiation. Our observations further strengthen the potential for pharmacological treatment of cognitive late-effects in childhood cancer survivors with the use of lithium. Current experiments aim at revealing the differences in genome-wide DNA methylation and associated gene expression after lithium treatment of control versus irradiated cells, as well as the role for TET3 in these events.

Funding Source: The Swedish Childhood Cancer Foundation, The Swedish Cancer Society, The Swedish Research Council.

Keywords: Epigenetics, Late effects, Brain tumor

Poster: 563

CRYOPRESERVATION AS A KEY TECHNOLOGY FOR NEURAL 3D CELL MODELS IN NEXT-GENERATION THERANOSTICS OF BRAIN PATHOLOGIES

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The biomedical impact of three-dimensional (3D) organoid cultures has been steadily increasing in recent decades. Organoids derived from human pluripotent stem cells (hiPSCs) represent model systems for human physiology and thus provide unique opportunities in biomedical research for diagnostics and disease modelling. Neural organoids even have the potential to serve as reprogramming and monitoring units in the interdisciplinary theranostic approach in the EU project GLADIATOR, when genetically modified. However, their generation is time consuming and they exhibit a limited shelf life. To enable stock keeping and secure a constant supply of the 3D cell system without batch-to-batch variations, organoid biobanks are key elements in biomedical research. So far, cryopreservation of biological material has only been satisfactorily realized for single cells in suspension. Main challenges for organoid cryopreservation are the inhomogeneous heat and mass transfer throughout the 3D system, causing harmful gradients and thus risking their structural integrity, which is essential for the maintenance of structure and function. In general there are two cryopreservation regimes, the conventional slow-rate freezing and the ice-free vitrification, both with advantages and disadvantages regarding organoid preservation that we comparatively analyse in this work. Mechanisms of cryo injuries were detected in early neural stem cells organoids, a homogenous precursor state of fully mature midbrain organoids. A scalable suspension-based bioreactor system is applied to reproducibly generate neural organoids, serving as basic 3D cell model for the conducted systematic studies. Quality controls for 3D cell models after cryopreservation are applied and might be extrapolated for different organoid parameters. Thus, a basis for the implementations of innovative high throughput cryopreservation concepts can be implemented.

Funding Source: This work has been funded under H2020-EU.1.2.1 grant agreement ID 828837.

Keywords: Cryopreservation, Neural 3D cell model, Theranostics

Poster: 564

A CONSISTENT GABAERGIC NEURON MODEL BY OPTIMISED REPROGRAMMING OF HUMAN IPSCS

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Neuronal circuits in the cortex consist of two main types of neurons, namely the glutamatergic principle excitatory cells and the GABAergic inhibitory neurons (INs). The inputs of INs provide cortical networks with the ability to balance spontaneous and evoked excitatory activities, and prevent runaway excitation. Abnormal IN function has been associated with a variety of neurological diseases, including schizophrenia, autism and Alzheimer's disease. For neuronal indications, fewer than 10% of findings derived from animal models can be translated to the clinic. Scalable approaches are needed to generate human in vitro models suitable for high content drug screening that consist of well-defined and pure populations of specific neurons, such as GABAergic neurons. We used our proprietary gene-expression technology, opti-ox, to tightly control the expression of ASCL1 and DLX2 and generate a pure population of GABAergic neurons from human iPSCs, at scale, within 14 days. A deep molecular characterisation of these neurons by immunocytochemistry, RT-qPCR and single cell RNA-sequencing revealed that the cultures consist of over 95% of GABAergic neurons expressing the classical marker genes GAD1, GAD2, VGAT, DLX1 as well as DLX2 and are positive for GABA. Functional annotation of genes enriched in the post-mitotic GABAergic neurons identified gene-sets significantly associated with several neurological disorders linked to INs, including schizophrenia, autism and ADHD. Anchoring our single cell RNA-seq data of the iPSC-derived GABAergic neurons on the Allen Brain single cell map of the human cortex showed that these neurons closely match with the VIP subtype of GABAergic neurons. A functional analysis by MEA demonstrated that they form neuronal networks and spontaneously fire action potentials within 2 to 3 weeks. Our highly consistent and synchronised opti-ox cellular reprogramming enables us to manufacture highly pure GABAergic neurons in large quantities in a reproducible manner. opti-ox reprogramming technology is being applied to a range of other neuronal subtypes and opens up novel avenues for the development of more reliable human in vitro models to support research and healthcare innovations.

Keywords: iPSC-derived neurons, disease model, GABAergic neurons

Poster: 565

THE AUTISM RISK FACTOR CHD8 IS A CHROMATIN ACTIVATOR IN HUMAN NEURONS AND FUNCTIONALLY DEPENDENT ON THE ERK-MAPK PATHWAY EFFECTOR ELK1

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The chromodomain helicase DNA-binding protein CHD8 is among the most frequently found de-novo mutations in autism spectrum disorder (ASD). Despite its prominent disease involvement, little is known about its molecular function in the brain. CHD8 is believed to be a chromatin regulator, but mechanisms for its genomic targeting are also unclear. By modeling human neuronal cell model carrying conditional CHD8 loss-of-function alleles, we characterized chromatin accessibility and transcriptional profiling that showed CHD8 is potent chromatin and transcription activator of its direct neuronal targets, including a distinct group of ASD genes with high correlation in human cortical neurons, implying they might belong to a functional disease module. We also found that CHD8-dependent chromatin remodeling was enhanced at ELK1 motif-containing CHD8 binding sites in a distinct directional pattern, demonstrating functional cooperativity between ELK1 and CHD8 on chromatin. These findings imply the involvement of the MAPK/ERK pathway in the pathogenesis of autism caused by CHD8 mutations.

Keywords: Autism, Chromatin, Stem cell-derived neurons

Poster: 566

RESCUE AXONAL DEGENERATION IN HUMAN STEM CELL MODELS OF HEREDITARY SPASTIC PARAPLEGIA WITH MITOCHONDRIAL FISSION INHIBITOR

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of inherited diseases characterized by spasticity and weakness of the leg and hip muscles. SPG11 and SPG48, two common recessive forms of HSP, are caused by mutations in SPATACSIN

and AP5Z1, leading to the degeneration of cortical motor neuron axons. Though mitochondrial dysfunction has been observed in HSP neurons, how human axons degenerate in HSP and whether targeting mitochondrial dysfunction can rescue disease phenotypes remain largely unclear. In this study, we dissect the role of mitochondria dysfunction in the degeneration of cortical neuron axons in SPG11 and SPG48. The patient iPSC-derived cortical PNs showed impaired axonal transport of mitochondria, accumulated neurite swellings and increased disease-related neurofilament release, which recapitulate disease-specific axonal degeneration. Moreover, these HSP cortical neurons exhibited reduced mitochondrial length, area, and mitochondrial membrane potential, implying mitochondrial dysfunction in axonal degeneration of HSP neurons. Interestingly, in patient iPSC-derived cortical neurons, there was a significant increase in the accumulation of abnormal neurofilament aggregations. Finally, treatment of a short peptide that inhibits mitochondrial fission mitigated mitochondrial dysfunction and abnormal neurofilament aggregations. Notably, the impaired axonal transport and degeneration of these neurons were rescued by the peptide. Taken together, our study reveals that mitochondrial defects underlie cytoskeleton disorganization and axonal degeneration of HSP neurons, highlighting the importance of targeting these pathologies therapeutically.

Keywords: Hereditary spastic paraplegia, Mitochondrial dysfunction, neural degeneration

Poster: 567

CRYOPRESERVATION OF BRAIN ORGANOIDS USING VITRIFICATION TECHNIQUE

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Cryopreservation at ultra-low temperature has been a valuable tool to preserve cells and tissues in different aspects of science. However, brain organoid cryopreservation remains a major challenge due to tissue complexity and the sensitivity of neurons to temperature shifts. Conventional cryopreservation techniques for stem cells and other organoid types (such as lung organoids) have not shown good results in cryopreserving brain organoids. We optimized the vitrification technique that has been extensively used to cryopreserve human embryos and oocytes to cryopreserve human brain (cortical) organoids. This technique starts with pre-equilibrating brain organoids in cryoprotectant solution, resulting in dehydration of the cells within the organoid and permeation with the cryoprotectant. The brain organoids are then exposed to a high concentration of cryoprotectant for a short period of time (~1min) and immediately immersed in liquid nitrogen. The high osmolality of the cryoprotectant results in complete dehydration of the cells. Using this technique, we successfully cryopreserved human brain organoids generated using two different methods and at different developmental stages. We thawed organoids and successfully maintained them in culture. We examined the cytoarchitectural integrity of thawed organoids by immunostaining with antibodies against markers of ventricular zone radial glia, proliferating and dying cells, and neurons. We found no significant differences between the vitrified brain organoids and control brain organoids grown in parallel that were not vitrified. In addition, the vitrified organoids demonstrated a growth rate comparable to the controls. Ongoing work is exploring the application of this technique to human brain organoids at different days of culture and sizes, and to different types of brain organoids (e.g., ganglionic eminence; thalamus). Our human brain organoid cryopreservation method provides a promising approach for bio-banking and should allow for the transfer of cryopreserved organoids between institutions for investigations of human brain development and genetic neurodevelopmental disorders.

Keywords: Cryopreservation, Vitrification, Brain organoid

Poster: 568

INVESTIGATING THE CELL-TYPE SPECIFIC ROLES OF FOXP1 IN THE DEVELOPMENT OF HUMAN NEOCORTEX USING BRAIN ORGANOIDS

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The molecular mechanisms underlying human cognition remain mostly unknown. Insights into the genes and pathways important for cognition will provide entry points into understanding cognitive disorders such as autism. De novo mutations in the gene encoding the transcription factor FOXP1 lead to a syndromic form of autism characterized by intellectual disability. Studies from our lab using Foxp1 cortical conditional knockout mouse models have shown that cortical lamination is altered and autism-relevant behaviors are affected. Recent findings in the human fetal brain show that FOXP1 expression is high in progenitor cells including the cell types (e.g. basal radial glia and basal intermediate progenitors) that are associated with human cortical expansion. Moreover, when overexpressed, human FOXP1 results in more progenitors than mouse Foxp1. Other genetic manipulation experiments have shown that knocking down Foxp1 at E13.5 affects results in cells unable to exit the intermediate progenitor (IP) stage. Therefore, I hypothesize that FOXP1 is important for proper generation of cortical projection neurons in the cortical plate (CP). I am testing this hypothesis using iPSC-derived human forebrain organoids and FOXP1 whole body knock-out mice as model systems of early neocortical development. So far, cortical lamination and BrdU birth dating experiments show signs of pre-mature differentiation. Proliferation of cells in the ventricular zone (VZ) also seem to be affected. Single nuclei RNA-seq technique shows changes in the regulation of genes involved in proliferation, differentiation and axonogenesis. Interestingly, different sets of genes seem to be regulated differently by FOXP1 in VZ, IP and CP. FOXP1 also seems to regulate a human specific gene, FAM182B, which is not expressed in rodents or non-human primates. Together, these data will inform our understanding of the role of FOXP1 in human brain development and how such mechanisms might be relevant to autism and human cognition.

Keywords: FOXP1, cortical projection neurons, intermediate progenitor cells

Poster: 569

INFINITY BIOLOGIX - A GLOBAL STEM CELL RESOURCE

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Infinity BiologiX maintains multiple NIH and non-profit stem cell repositories in including 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, fibroblasts and cryopreserved lymphocytes from more than 1000 subjects, including a GMP grade iPSC cell line. These cell lines are available to academic and for-profit researchers worldwide. Since its inception in 1998, RUCDR Infinite Biologics has provided the global scientific community with the highest quality biomaterials, technical consultation, and logistical support. In 2011, with rising interest in induced pluripotent stem cells (iPSC) models of human development and disease progression and for drug screening and toxicology testing RUCDR began offering a wide range of stem cell services. These include source cell and iPSC banking, iPSC generation, iPSC gene editing and source cell and iPSC distribution. On August 17th, 2020, Rutgers University completed the sale of RUCDR and Infinity BiologiX was created. As Infinity BiologiX, we have the same core mission, advancing research globally. All iPSC generated and distributed by IBX include the following minimum set of QC assays - sterility (including mycoplasma), identity, expression of the stemness marker alkaline phosphatase and expression of the stemness markers Oct4 and Tra-1-60. In addition to these assays, many cell lines have been karyotyped, been shown to have lost the expression of the reprogramming factors and have been assayed for differentiation into all 3 germ layers. All cell lines are distributed with a certificate of assurance, guidelines for culturing the cells and any technical support required to help a client meet their research goals. In addition to the repositories housed at Infinity BiologiX, we also offer a full range of stem cell services, including CRISPR gene editing of iPSC all based on the best practices in the field.

Funding Source: NINDS U24NS095914 NIMH U24MH068457

Keywords: iPSC Biorepository, Neuro-disease, Neural disease modeling

Poster: 570

GENERATION OF ISOGENIC IPSC LINES FOR RARE DISEASE MODELING VIA CRISPR/CAS9 GENE EDITING

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The integration of human induced pluripotent stem cell (hiPSC) and CRISPR/Cas9 gene editing technologies has created new possibilities to investigate rare genetic disorders, including genotype-phenotype relationships in disease-relevant cell types. The NYSCF Global Stem Cell Array® team is collaborating with numerous patient-driven rare disease foundations to expand our community repository of hiPSCs from patients with rare diseases, including Dravet Syndrome, hereditary hemorrhagic telangiectasia (HHT), Down Syndrome, Ogden Syndrome, infantile neuroaxonal dystrophy (INAD), Juvenile Batten (CLN3) Disease, NGLY1 Deficiency, and many more. As the single pathogenic mutation is often known for these diseases but its functional impact remains unclear, we have been generating isogenic disease and control-hiPSC line sets via CRISPR/Cas9. Recent examples include: INAD is a type of PLA2G6-associated neurodegeneration (PLAN). The inherited neurodegenerative disorder is characterized by abnormalities of nerve endings within the brain, spinal cord and peripheral nerves. In collaboration with the INADCure foundation, we corrected the homozygous pathogenic PLA2G6 mutation p.Arg70* in an INAD patient iPSC line. CLN3 Disease is a rare neurodegenerative disorder that develops between the ages of 5 and 10, caused by mutations in the CLN3 gene. In collaboration with the Beyond Batten Disease Foundation (BBDF), we previously generated iPSCs from several families. We are currently generating isogenic pairs for iPSC lines with E13 p.Arg334His or p.Val330Phe or p.Gly154Alafs*29, Val155_Gly264del. NGLY1 Deficiency is a congenital deglycosylation disorder that affects multiple body systems and delays developmental milestones. In collaboration with the Grace Science Foundation (GSF), we generated affected patient-derived iPSC lines and isogenic controls (for NGLY1 p.Glu311Lys and NGLY1 p.Gln631Serfs*7, respectively). We are now using these isogenic pairs to dissect disease mechanisms via differentiation into disease-relevant cell types to gain valuable insights into the impacts of these pathogenic variants. To access our rare disease patient, isogenic control, and healthy control hiPSC lines, please visit the NYSCF repository website at nyscf.org/repository.

Keywords: iPSC, Rare Disease, CRISPR, NGLY, CLN3, PLA2G6, isogenic lines

Poster: 571

HUMAN IPSC-DERIVED MICROGLIA FOR NEUROINFLAMMATORY DISEASE MODELING

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Microglia contribute to a range of neuroinflammatory and neurodegenerative disorders, but scientists have long suffered from a lack of quality in vitro models for these cell types. We have developed a robust and scalable method to produce highly pure, functionally validated, and ready-to-use microglia from human induced pluripotent stem cells (iPSC). These microglia show greater than 97% purity as measured by immunostaining for IBA1 and TMEM119. These cells can be cryopreserved, thawed, and cultured in defined maintenance media with or without astrocytes or neurons for prolonged culture. We have used high-content imaging for phenotypic characterization of these models, including observing a re-distribution of inflammasome proteins (NLRP3 and ASC) from diffuse and cytoplasmic to organized punctate structures. This inflammasome formation could be blocked by the inhibitor, MCC950. These data show functionally relevant and measurable phenotypes in these human iPSC-derived microglia may be suitable for modeling neuroinflammatory diseases and in drug-screening assays.

Keywords: microglia, neuroinflammation, iPSC

Poster: 572

TROPISM OF SARS-COV-2 FOR DEVELOPING HUMAN CORTICAL ASTROCYTES

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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 a significant proportion 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 primary human cortical tissue and stem cell-derived cortical organoids from a range of developmental stages. We find significant and predominant infection in cortical astrocytes in both primary and organoid cultures, with minimal infection of other cortical populations. Infected astrocytes had a corresponding increase in reactivity characteristics, 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 DPP4 and CD147. Inhibition of DPP4 reduced infection and decreased indicators of cell stress. We find tropism of SARS-CoV-2 for human astrocytes independent of ACE2, resulting in reactive gliosis-type injury. Our study provides evidence of direct infection of a specific human neural cell type with implications for the vulnerability in the developing brain and the potential for neural infection in postnatal life. The range of COVID-19 associated neurological symptoms including dizziness, seizures, and cognitive difficulties, may reflect the involvement of astrocytes which are vital to global brain homeostasis and function.

Keywords: SARS-CoV-2 Tropism, Astrocytes, Human Cortex

Poster: 573

GENETIC BACKGROUND DRIVES SEVERITY OF 16P11.2 CNV PHENOTYPE IN HUMAN BRAIN ORGANOIDS

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Deletion and duplication copy number variants (CNVs) in the 16p11.2 genomic region are associated with a several neurodevelopmental disorders, such as autism spectrum disorder and schizophrenia. This genomic locus harbors 29 protein-coding genes, and their causative role in neurodevelopmental disorders is unclear. 16p11.2 pathogenic rearrangements are a human-specific genetic phenomenon, which makes it challenging to recapitulate human phenotypes in mice. Here we generated human brain organoids from 21 patient and control iPSC lines to model 16p11.2 copy number variation. Profiling of 185,645 cells by single-cell RNA-seq revealed cell type-specific differential expression of both cis (within the CNV) and trans (outside the CNV) genes in 16p11.2 hemi-deletion organoids. We identified and validated multiple trans genes with altered expression that are related to neuronal cell-cell adhesion and projection growth, as well as genes that were previously associated with neurodevelopmental disorders. In addition, we used CRISPR technology to engineer the 16p11.2 hemi-deletion into a line with genetic background of a neutral polygenic risk score (PRS). Organoids generated from these isogenic lines did not show differential expression of trans genes when comparing deletion-bearing to wild-type organoids. Our data underscores the importance of genetic background in enhancing the phenotype of the pathogenic 16p11.2 CNV and establishes a link between the rare 16p11.2 CNV and the common risk genes of neurodevelopmental disorders.

Keywords: brain organoids, neurodevelopmental disorders, single cell RNA-seq

Poster: 574

ANTAGONISM OF THE RNA BINDING PROTEIN SYF2 MITIGATES NEURODEGENERATION AND TDP-43 PROTEINOPATHIES IN AMYOTROPHIC LATERAL SCLEROSIS BY INCREASING RNA EXPORT

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron degeneration. A major pathological feature in 97% of ALS patients is the aggregation and cytoplasmic mislocalization of the RNA binding protein TDP-43 in the brain and spinal cord. Therefore, the identification of strategies to minimize aggregation and cytoplasmic mislocalization is critical for alleviating TDP-43 proteinopathies. To identify compounds that rescue the survival of induced motor neurons (iMNs) generated from iPSCs derived from multiple ALS patients with known or unknown causal mutations, we screened an FDA-approved library of 2000 drugs and 1800 tool compounds. We found that steroids potently extended iMN survival. Bioinformatic analysis of existing RNA profiling data on FDA approved drugs and genetic knockdowns using Connectivity Map database identified SYF2 suppression as inducing similar gene expression changes as steroid treatment. Treatment of C9ORF72 ALS and sporadic ALS iMNS with ASOs targeting SYF2 resulted in a potent reduction in neurodegeneration. SYF2 is a pre-mRNA splicing/RNA export protein that is predominantly localized to the nucleus. Given that other studies have shown that SYF2 interacts with RNA export complexes and that RNA binding prevents TDP-43 aggregation, we hypothesized that SYF2 is a negative regulator of RNA export. To test this hypothesis, iMNs treated with a negative control or SYF2 ASO were pulsed with 5-Ethynyl Uridine (EU) to label nascent RNA. SYF2 ASO treatment increased cytoplasmic RNA levels and reduced the nuclear:cytoplasmic ratio of RNA in iMNs. In addition, SYF2 ASO treatment reduced cytoplasmic mislocalization of TDP-43. To assess the impact of SYF2 suppression on cytoplasmic TDP-43 aggregation, iMNs were transduced with a Cry-TDP-43 mcherry fusion protein that forms aggregates in the presence of blue light. SYF2

suppression reduced the formation of cytosolic aggregates. The efficacy of SYF2 suppression in vivo was also assessed in a TDP-43 transgenic mouse model of ALS. We found that SYF2 suppression potently rescued motor deficits. In conclusion, SYF2 suppression rescues ALS neurodegeneration by a novel mechanism consisting of RNA export, decreasing cytosolic TDP-43 oligomerization, and restoring nuclear TDP-43 levels.

Keywords: RNA export, TDP-43, SYF2

Poster: 576

DEVELOPING ANTISENSE OLIGONUCLEOTIDE THERAPY FOR PCDH19 CLUSTER EPILEPSY USING A HUMAN BRAIN ORGANOID MODEL

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PCDH19 Cluster Epilepsy (PCE) is caused by variants in the PCDH19 gene located on the X-chromosome. It exclusively affects females and mosaic males while male carriers are spared. This unique inheritance pattern may be explained by "cellular interference" caused by impaired interactions of cell populations expressing only wildtype PCDH19 with those expressing only mutant PCDH19 during brain development due to random X-inactivation. These impaired interactions lead to segregation of each cell population. To examine cell segregation phenotypes in human embryonic stem cell (hESC)-derived brain organoids, we generated CRISPR homozygous PCDH19 knockout H9 female hESCs and generated a "virtual PCE patient (PCDH19+/PCDH19-)" model in which isogenic RFP/GFP-labeled H9 cells with a HA-FLAG-tagged PCDH19 (here denoted wild type, WT) allele are mixed with GFP/RFP-labeled knockout cells, providing a reliable system to model mosaic PCDH19 expression. Then we generated brain organoids after 1:1 mixing of knockout and WT cells (PCE model), WT and WT cells, or knockout and knockout cells (asymptomatic carrier model). We observed robust cell segregation in VZ/SVZ-like regions only in the PCE brain organoids, which also displayed abnormal localization of PCDH19 and N-cadherin (NCAD), as well as abnormal early cortical lamination. None of these phenotypes were seen in brain organoids generated with mixing only wild type or only knockout labeled cells. Based on these results, we hypothesized that attenuating wildtype PCDH19 expression will relieve the imbalance between wildtype and mutant PCDH19-expressing cells, modeling an asymptomatic carrier, and thereby prevent PCE-related brain abnormalities. We identified two antisense oligonucleotides (ASOs) that appear to substantially reduce PCDH19 protein levels. Work is ongoing in the PCE brain organoid model to determine whether attenuating wildtype PCDH19 expression with ASOs alleviates the cell segregation and other neurodevelopmental phenotypes in vitro. In future studies, positive ASO hits will be validate in vivo using a mouse PCE model developed in our laboratory. This therapeutic strategy has the potential of preventing or halting the progression of seizures and cognitive impairment in patients with PCE.

Keywords: PCDH19 Cluster Epilepsy, antisense oligonucleotide, human brain organoid

Poster: 577

THE ROLE OF CD47 IN BRAIN OVERGROWTH AND 16P11.2 DELETION SYNDROME

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Copy number variation (CNV) at the 16p11.2 locus is associated with Autism spectrum disorder (ASD). People who have 16p11.2 deletion syndrome tend to have larger head circumferences (macrocephaly), with disproportionate enlargement in both gray and white matter volume. Individuals with ASD and macrocephaly have more severe behavioral and cognitive problems than those with ASD and normal head circumferences. Prior work has documented a very strong cross-sectional and temporal association between macrocephaly and ASD symptoms, suggesting that understanding the mechanisms regulating macrocephaly could help identify a window of opportunity for intervention. Here, we use patient-derived hiPSCs from 16p11.2 deletion carriers to interrogate the underlying mechanisms contributing to gray and white matter enlargement. We differentiate the iPSCs into neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs) and investigate the hypothesis that brain enlargement may be due to improper cellular elimination. Under normal conditions, classical 'eat me' and 'don't eat me' signaling mechanisms associated with phagocytosis maintain cellular homeostasis across diverse tissue types. CD47 (a 'don't eat me' signal) protects normal cells from getting cleared, but can become overexpressed in many types of cancer cells, preventing engulfment. CD47 plays a role in many disorders associated with an overproduction of cells and/or cell removal, including cancer, atherosclerosis, and fibrotic diseases. NPCs derived from iPSCs of autistic individuals with macrocephaly have increased proliferation relative to controls. Therefore, we hypothesized that CD47 may be involved in these disorders. We find that CD47 is overexpressed in NPCs and OPCs derived from 16p11.2 deletion carriers, leading to reduced phagocytosis by macrophages and microglia. The 16p11.2 deletion NPCs and OPCs have increased cell surface expression of calreticulin (CRT, a pro-phagocytic 'eat me' signal), indicating that these cells should be eliminated but are not due to high levels of CD47. Treatment with a CD47 blocking antibody restores phagocytosis of 16p11.2 deletion NPCs and OPCs to control levels. We thus document a novel role for CD47 in psychiatric disorders associated with brain overgrowth.

Keywords: 16p11.2 deletion, iPSCs, macrocephaly

Poster: 578

MUTATIONS IN ASD RISK GENES RESULT IN ALTERED GENERATION OF CORTICAL NEURONS IN HUMAN BRAIN ORGANOIDS.

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3D organoids hold unprecedented promise for basic understanding of human brain development and disease; however, their use as experimental systems has been limited by their poor characterization and inherent reproducibility. We recently showed that a newly optimized organoid model pre-patterned to form the dorsal forebrain can achieve highly reproducible generation of the rich cellular diversity of the developing human cerebral cortex, demonstrating that the establishment of terminal cell identity is a highly constrained process that can emerge outside the embryo. We leveraged this reproducible cortical organoid model to identify cell type-specific developmental abnormalities associated with haploinsufficiency in the ASD risk gene SUV420H1 (KMT5B). By performing comprehensive single cell RNA-sequencing on individual organoids collected at different developmental stages, we found that within a defined period of early cortical development, SUV420H1 haploinsufficiency causes accelerated development of a specific neuronal cell type. This work shows that reproducible cortical organoids, combined with high-throughput single cell genomic methods, are invaluable systems for unbiased identification of pathogenic mechanisms in neurodevelopmental disorders, and paves the way for further efforts to analyze a larger spectra of ASD risk genes in organoids, to understand whether they converge on shared disease pathology.

Keywords: Brain organoids, Autism spectrum disorder, Single cell RNA sequencing

Poster: 579

RETROMER STABILIZING COMPOUNDS RESCUE ENLARGED ENDOSOME PHENOTYPES IN HIPSC-DERIVED MODELS OF SPORADIC ALZHEIMER'S DISEASE

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The SORL1 gene encodes for the protein SorLA, a sorting receptor involved in retromer-related endosomal traffic. Many SORL1 genetic variants increase Alzheimer's disease (AD) risk, and rare loss-of-function truncation mutations have been found to be causal of AD. Previous work has shown that SORL1 depletion results in enlarged early endosomes in hiPSC-derived neurons. We used CRISPR/Cas9 technology to insert SORL1 sporadic AD-risk variants in human induced pluripotent stem cells (hiPSCs) to test the hypothesis that risk variants in the VPS10 domain of SORL1 (SORL1 VPS10 variants) contribute to AD pathogenesis by leading to dysfunction in endosomal trafficking. We report that SORL1 VPS10 variants in hiPSC-derived neurons lead to early endosome enlargement, a cellular phenotype that is indicative of 'traffic jams' and is now considered a hallmark cytopathology AD. We determine that the retromer stabilizing compound TPT-260 reduces early endosome enlargement in SORL1 VPS10 variants. Our data, together with recent findings, underscores how sporadic AD pathways that regulate endosomal trafficking, and autosomal-dominant AD pathways that regulate APP cleavage, independently converge on AD's defining cytopathology. Moreover, demonstrating a partial rescue of cellular phenotypes in SORL1 VPS10 variants will contribute to the development of new and precision treatments for AD.

Keywords: Alzheimer's Disease, Endosomal Trafficking, Gene Editing

Poster: 580

DERIVATION OF OLFACTORY PLACODAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Olfactory dysfunction, or loss of smell, is estimated to occur in 5.8 to 11.6 million people in North America. Olfactory sensory neurons are receptors in the nasal cavity that detect odors from the environment and transmit odorant information to the brain. Loss of smell can occur from degeneration of olfactory sensory neurons and is often the first symptom experienced in neurodegenerative diseases. Cell based therapies have the potential to treat olfactory dysfunction through replacement of olfactory progenitors, also known as olfactory placodal cells. Human pluripotent stem cells (hPSCs) have the ability to differentiate into any cell type in the body; therefore, they provide an invaluable tool for cell replacement therapies. The first step towards their therapeutic use in olfactory dysfunction is to create a protocol to direct the differentiation of hPSCs into olfactory placodal cells following a series of molecular cues that recapitulate characteristics of their natural development. We utilized a combination of various small molecules and growth factors to generate olfactory placode. Our key findings indicate that timed exposure to small molecule inhibitors of bone morphogenetic protein (BMP), wingless/integrated protein (WNT), and retinoic acid receptor (RAR) signaling pathways promote olfactory placode development with neural potential after 24 days of hPSC differentiation. The addition of TGFα and FGF8 further elevated olfactory placode gene expression. Furthermore, we report the generation of olfactory marker protein positive neurons from hPSC-derived olfactory placodal cells at day 30 of differentiation. All together our results will lead to the development of new therapies for diseases of the olfactory tissue and promotion of sensory nerve regeneration.

Funding Source: This research was supported by the National Center for Advancing Translational Science of the NIH, grant award TL1TR001119.

Keywords: hPSCs, differentiation, olfactory

Poster: 581

EMERGENCE OF SYNAPTIC MODULES DURING EARLY CORTICAL DEVELOPMENT

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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 the physiological and morphological features of early intracortical connectivity in the developing human cortex during the second trimester, when synaptogenesis is thought to begin. 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 were observed in the synaptic modules. Furthermore, local synaptic modules are regulated by serotonin signaling through 5-HT2A receptors. Understanding the development of early intracortical circuits will inform in vitro models of human brain development and may shed light on the etiology of neurodevelopmental disorders.

Keywords: Brain Development, Synaptic Development, Serotonin Modulation

Poster: 582

MODELING NEURAL TUBE DEFECTS WITH SELF-ORGANZING SINGLE ROSETTE CORTICAL SPHEROIDS (SOSRS), A STRUCTURALLY REPRODUCIBILE BRAIN ORGANOID TECHNOLOGY

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The field of brain organoid research is complicated by structural variability with multiple rosette structures per organoid. We have developed a new organoid technique that generates self-organizing, single-rosette brain organoids (SOSRS) with a reproducible diameter and cell diversity. Unlike previous techniques which pattern a 3D embryoid body, we pattern a 2-dimensional monolayer followed by induction of neurulation into 3-dimensional self-organizing single rosette spheroids (SOSRS). These SOSRS follow many hallmarks of cortical development and can be maintained for over 150 days in culture. SOSRS have an extremely reproducible diameter and growth rate, and single-cell RNA-sequencing shows the individual SOSRS are highly similar in cell type diversity. In order to provide evidence of neurulation, we blocked proteins in the apical constriction pathway (Rho-kinase and non-muscle myosin) resulting in loss of lumen circularity, increased apical endfeet diameter, and enlarged lumens. Given the high rate of neural tube defects (NTDs) in human pregnancy and the lack of appropriate human models, we began testing the effects of other known human teratogens on SOSRS neurulation. Valproic acid, an antiseizure medication known to cause NTDs like spina bifida, resulted in dysmorphic SOSRS and enlarged lumens in a dose-dependent manner beginning at 100 µM, a pharmacologically relevant dose of serum free valproic acid. Previous studies have indicated that valproic acid teratogenicity is the result of either HDAC or folic acid pathway inhibition. To test these hypotheses, we added the more specific inhibitors trichostatin-A (HDAC inhibitor) and aminopterin (folic acid pathway inhibitor). Neither of these compounds resulted in a phenotype similar to valproic acid, indicating another mode-of-action resulting in NTD formation. In summary, we have shown that our structurally reproducible brain organoid model with single rosettes allows for detailed investigation of altered structural brain developmental phenotypes, even at the stage of neurulation. The ability to model NTDs in a 3-D human cell culture model will be useful for teratogenic drug screening and understanding the mechanisms underlying altered neurodevelopment.

Keywords: Brain organoids, Neural tube defects, Valproic acid

Poster: 583

DERIVATION OF A HUMAN BRAIN ORGANOID WITH MICROGLIA

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3D brain organoid culture derived from induced pluripotent stem cells (iPSCs) provides an important alternative in vitro tool for research of human brain development and pathogenesis. However lacking the incorporation of glial cells, especially microglial cells, in the human brain organoids is still the main hurdle for 3D model of neuroinflammation. Current protocols to addressing this issue either incorporated fully differentiated microglia into the brain organoids, or started the microglial differentiation together with the neural induction from the early stage of 3D organoid formation. The first approach misses the stage when microglial differentiation interacts with the adjacent neural environment and the later approach involves technical difficulty to produce consistency among the final organoids in terms of the quantity and quality of microglia. Thus, brain organoid models which have incorporated microglia to study the early interactions between microglial and neuronal development are needed. Here, we derived CD34+ cells first from human iPSCs, then incorporated the CD34 cells into iPSC-derived embryoid bodies to make brain organoids. Using immunostaining and flow cytometry analysis, we confirmed that CD34 cells incorporated into the 3D organoids which eventually developed into brain organoids with both Iba1+ microglia and beta-III tubulin+ neurons. Compared to brain organoids without CD34 incorporation, this methods produced microglial incorporation significantly in the brain organoids. This novel 3D organoid model consisting both microglial and neural developments can be used to study the early interactions between the innate immune and nervous system development and potentially as a model for neuroinflammation.

Funding Source: This work is supported by NIH/NINDS intramural research fund.

Keywords: brain organoid, microglia, CD34

Poster: 584

FUNCTIONAL AND HOMOLOGOUS HUMAN 3D BRAIN ORGANOIDS FOR PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE MODELING

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Human brain organoids derived from human pluripotent stem cells (hPSCs), including patient iPSCs, provide promising in vitro human micro-tissues models to study brain development, CNS disease mechanisms, as well as drug toxicity/efficacy. Due to the lack of stability, structure consistency and mature function, 3D brain organoids generated by most methods are not suitable models for disease study or drug testing. We have developed a robust and stable method to produce homologous functional 3D cerebral and midbrain organoids. These cerebral organoids have similar development stage signatures and cell compositions mimicking human forebrain, including 6 cortical layer neurons, astrocytes and oligodendrocyte progenitor cells. They have synchronized spontaneous firings starting at 20 days of culture, significant responses to NMDAR agonist and antagonist at about 40 days, and display periodic and rhythmic brain activity after 60 days in vitro, detected by calcium imaging or multiwell microelectrode array (MEA). We further found that the Amyloid-β (Aβ) oligomers and alpha synuclein (α-syn) aggregates could induce pathological features of Alzheimer's disease (AD) or Parkinson's disease (PD) in these homologous cerebral organoids and midbrain organoids, respectively. These novel models will provide great aid to the mechanism study and drug discovery of CNS diseases.

Keywords: 3D brain organoids, Parkinson's disease modeling, Alzheimer's disease modeling

Poster: 585

PLATFORM TO DIFFERENTIATE SENSORY NEURONS AND ENRICH SPECIFIC SUBTYPES FROM THE SAME HPSC CULTURE

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Sensory neurons (SNs), part of the peripheral nervous system, convey sensations such as pain, temperature, pressure, and limb movement/position to the central nervous system. They differentiate from migratory neural crest cells (NCCs) that localize to dorsal root ganglia (DRG). The three SN subtypes (nociceptors, mechanoreceptors, and proprioceptors) arise from two distinct waves of NGN2- and NGN1-expressing NCCs. Despite previous reports, to date no protocol is available allowing the generation of all three SN subtypes at high efficiency and purity from human pluripotent stem cells (hPSCs). We developed a versatile method using chemically defined to generate all three SN subtypes from the same starting population in a proportion similar to human DRG. Our SNs go through all the developmental stages (neural plate border, pre-migratory, and migratory NCCs) as measured by expression of specific markers by RT-qPCR. Approximately 70% of all cells are BRN3A+ SNs, which are electrically active and respond to specific stimuli. Furthermore, our SNs express mature marker, such as the Glutamate transporters (Glut1-3), members of the transient receptor family (TRPV1 and TRPV2), temperature sensitive receptors TRPM8 and TRPA1 (present in nociceptors), the mechanically activated K+ channels TRK1 and TRAAK, as well as PIEZO2 (present in mechanoreceptors), and SPP1 and Parvalbumin (present in proprioceptors). Also, our SNs can be dissociated and replated maintaining their electrical activity, suggesting that they can be used as a model to study axotomy. Finally, we identified two methods to enrich for specific SN subtypes. Similar to physiological conditions, our NCCs show peak expression of NGN1 and NGN2 in two distinct waves, which can be used to guide their differentiation towards nociceptors (NGN1) or mechanoreceptors (NGN2). Alternatively, we show that immunoapnning, a gentle method that allows purification of cells based on surface proteins, can be used to isolate SNs based on their expression of membrane-bound TRK proteins. Our protocol can also be used to model peripheral neuropathies, such as familial dysautonomia, using patient-derived iPSCs. Thus, our work allows the modeling of human disorders affecting SNs, the search for treatments, and the study of human development.

Keywords: neural crest, sensory neurons, disease modeling

Poster: 586

HUMAN PLACENTA DERIVED EXOSOMES (PEXO) PROVIDE THERAPEUTIC BENEFIT IN A TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) ISCHEMIC STROKE RAT MODEL

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The complications of ischemic stroke can be severe and debilitating. Current approved treatments for stroke are limited and have an extremely short window for therapeutic intervention. Recent efforts in the development of exosomes derived from various sources as potential therapeutics have expanded into treatment of stroke. Placental exosomes (pExo) originate from an organ that has been shown to support fetal brain development during pregnancy (1) and should retain key functions of the placenta in immunoregulation and regeneration during pregnancy. Here, we report the investigation of the therapeutic effects of pExo on ischemic stroke in a transient MCAO rat model. Sprague Dawley rats were treated with vehicle (Phosphate-Buffered Saline) or 100µg of pExo intravenously at 1, 6 and 11 days after induction of ischemic stroke by transient MCAO. Neurological and motor functions of rats were evaluated using the modified Neurological Severity Scale (mNSS) and several behavior tests, respectively. At study termination, the infarct volume was measured by Hematoxylin and Eosin (H&E) staining. In addition, neurogenesis was examined in the subventricular zone (SVZ) of the lateral ventricles and the hippocampus by doublecortin (DCX) staining. As early as 7 days post MCAO, pExo-treated rats showed significant improvements in neurological function ($p<0.001$) and motor function ($p<0.01$) compared to vehicle-treated rats, and continued improvement was observed until study termination on Day 35. Furthermore,

a significant reduction in infarct volume was observed in the pExo-treated rats compared to the vehicle control ($p<0.05$). A significant increase of DCX+ cells in the SVZ ($p<0.001$) as well as hippocampus ($p<0.01$) was detected in pExo-treated rats, indicating that pExo treatment enhanced neurogenesis. Our results demonstrate that pExo treatment alleviates neurological and motor function deficits induced by ischemic stroke, and pExo-mediated functional recovery is correlated with reduced infarct volume and enhanced neurogenesis. These results support further development of pExo as a potential therapy for the treatment of stroke.

Keywords: Exosomes, Placenta, Stroke

Poster: 587

ALTERED NEURONAL PHYSIOLOGY, DEVELOPMENT, AND FUNCTION ASSOCIATED WITH A COMMON CHROMOSOME 15 DUPLICATION INVOLVING CHRNA7

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While copy number variants at chromosome 15q13.3 cause neuropsychiatric disorders with variable penetrance, the basis of this phenomenon remains uncharacterized. Here, induced pluripotent stem cell models were generated from first-degree relatives with 15q13.3 duplication involving CHRNA7, including a boy with autism (affected proband-AP) and his clinically unaffected mother (UM), and compared to unrelated controls lacking this duplication. AP-derived models exhibited unique neurodevelopmental anomalies, including enhanced progenitor proliferation but impaired neuronal differentiation, maturation, and migration, and increased endoplasmic reticulum stress. The latter two phenotypes were pharmacologically reversed. Gene expression in related pathways was reduced in the AP but upregulated in the UM model, suggesting molecular compensation during neurodevelopment. By contrast, AP-and UM-derived neurons shared functional anomalies, including increased action potential firing and elevated cholinergic activity, consistent with increased CHRNA7 channel activity. This work defines diagnosis-associated phenotypes and shared anomalies related to HRNA7duplicationand potential contributors to its variable phenotypic penetrance.

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Keywords: Induced pluripotent stem cells, cortical neurons, chromosome 15q13.3 duplication in CHRNA7

Poster: 674

MICRORNA DYSREGULATION AS A DRIVER OF WNT ACTIVATION AND MOTOR NEURON DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Underlying mechanisms driving motor neuron (MN) degeneration in the fatal disease Amyotrophic Lateral Sclerosis (ALS) remain poorly understood due to complex disease heterogeneity and inaccessibility of diseased cell types. Consequently, development of effective treatments remains elusive. Recent studies identify unifying disruption to key MN homeostatic functions such as RNA processing, in both sporadic and familial ALS (sALS/fALS). It is yet unclear how such defects drive MN degeneration in ALS. In this study, we deploy patient-derived iPSC disease modelling to investigate the mechanisms driving ALS-associated MN degeneration. Patient-derived iPSCs harbouring mutations in the ALS-associated gene FUS were differentiated into motor neurons alongside isogenic and independent healthy control cells. We performed detailed phenotypic and transcriptomic analyses of iPSC-derived MNs, alongside computational analyses of existing ALS datasets to discover RNA perturbations and key signalling pathways that drive neurodegeneration. As a common feature of fALS and sALS, we explored disruption to microRNA networks in our model. We identify downregulation of a MN-specific microRNA, and identify this microRNA as a regulator of a key signalling pathway called WNT. Suppression of WNT improves MN survival, highlighting its role as a driver of MN degeneration. Importantly, we uncover that these molecular defects are also observed in sporadic ALS MNs, highlighting the shared dysregulation of RNA metabolism in familial and sporadic ALS. These findings expose WNT signalling as a driver of MN degeneration in ALS. In FUS-ALS MNs this is, at least in part, driven by FUS-mediated disruption to microRNA biogenesis, highlighting the importance of RNA processing in familial and sporadic ALS.

Keywords: Neurodegeneration, MicroRNA, WNT

Poster: 742

EFFICIENT GENERATION OF LUNG PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Efficient derivation of functional proximal or distal airway organoids from human pluripotent stem cells (hPSCs) would provide valuable in vitro models for studying lung development and respiratory diseases, and would facilitate future regenerative cell-based or gene therapies for complex respiratory disorders. To standardize the generation of hPSC-derived airway organoids, we developed STEMdiff™ Lung Progenitor Kit, a serum-free kit for the efficient differentiation of human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines into lung progenitor cells. The iPS (BU3-NGST, WLS-1C) and ES (H1, H7 and H9) cell

lines previously maintained in mTeSR™1 or mTeSR™ Plus were seeded as clumps onto Corning® Matrigel®-coated 96- or 24-well plates in mTeSR™1 or mTeSR™ Plus to generate monolayer cultures. One day after seeding, the medium was switched to STEMdiff™ Lung Progenitor Kit medium. The cells were then directed through 14 days of differentiation to sequentially generate definitive endoderm, anterior foregut endoderm, and lung progenitor cells. On day 14, differentiation was assessed for the expression and localization of lung progenitor markers (NKX2.1 or CPM) via immunostaining and flow cytometry. The lung progenitor cells coexpressed NKX2.1 and CPM, and are negative for the off-target markers PAX8 (thyroid) and FOXG1 (forebrain). We further demonstrated that the hPSC-derived lung progenitor cells are able to differentiate downstream into SPC positive alveolar spheroids. In summary, STEMdiff™ Lung Progenitor Kit supports efficient generation of bipotent lung progenitors and is reproducible across multiple hPSC lines.

Keywords: Lung progenitor, hPSC, NKX2.1

Poster: 954

HEMOPHILIA B DISEASE MODELING USING PATIENT-SPECIFIC IPSC: IN VITRO AND IN VIVO RECOVERY OF FIX CLOTTING ACTIVITY

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Hemophilia B (HB) is an X-linked monogenic disease characterized by reduced activity of circulating clotting factor IX (FIX), synthesized by hepatocytes. Current treatment based on regular intravenous injections of FIX is very restrictive, costly, and only palliative. Gene therapy trials show promising results but not all patients are eligible and their long-term efficacy is still unknown. It is thus important to explore other therapeutic strategies. To demonstrate the feasibility of a 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 bearing the Padua mutation at the AAVS1 safe harbor locus as a generic approach. Non-corrected and corrected hiPSCs were differentiated into hepatocytes under both 2D and 3D culture systems. Differentiated cells expressed hepatocyte-specific markers such as HNF4α, ALB and ATP7B. They exhibited signs of epithelial polarity, as shown by the lateral membrane staining 207 of ZO-1, occludin, and claudin-1. Immunostainings highlighted improved maturation of the cells issued from the 3D versus the 2D protocols, as reflected by the disappearance of AFP in 3D spheroids, and the expression of mature hepatocyte markers such as the cytochromes P450 3A4, the high-density lipoprotein receptor (scavenger receptor B1), the cytokeratin 8 and the connexin-32. The 3D-differentiated cells acquired complex polarization highlighted at the biliary pole of the hepatocytes by the expression of MDR3 as well as the bile salt export pump, an efflux transporter that plays an important role in eliminating bile salts from hepatocytes into the bile canaliculi. In vitro studies showed that FIX was produced by the differentiated cells in both 2D and 3D culture systems. However, only the 3D differentiation approach allowed the post-translational modifications occurring in fully mature hepatocytes, which permitted us to detect in vitro the FIX activity. Finally, we assessed the in vivo therapeutic efficacy of this approach using a mouse model of HB. Immunohistochemistry analyses indicated good cell engraftment and the FIX activity detected in the plasma of transplanted animals confirmed rescue of the bleeding phenotype.

Keywords: Hemophilia B, disease modeling, spheroids

FRIDAY, JUNE 25

00:00 - 1:00 EDT

POSTER SESSION 6

CELLULAR IDENTITY

Poster: 601

UNRAVELLING THE CHROMATIN LANDSCAPE AND ENHANCER LOGIC MEDIATING SPATIOTEMPORAL PATTERNING OF EARLY MOUSE CARDIOVASCULAR PROGENITORS

Swedlund, Benjamin[1], Lin, Xionghui[2], Guibentif, Carolina[3], Ghazanfar, Shila[4], Paulissen, Catherine[2], Achouri, Younes[5], Calonne, Emilie[6], Dubois, Christine[2], Mansfield, William[7], Zaffran, Stéphane[8], Marioni, John[4], Fuks, François[6], Göttgens, Berthold[9], Lescroart, Fabienne[8], Blanpain, Cédric[2]

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The mammalian heart arises from various populations of Mesp1-expressing cardiovascular progenitors (CPs) that are specified during the early stages of gastrulation. Mesp1 acts as a master regulator of CP specification and differentiation. However, how Mesp1 regulates the chromatin landscape of nascent mesodermal cells to define the temporal and spatial patterning of the distinct populations of CP remains unknown. Here, by combining ChIP-seq, RNA-seq and ATAC-seq during mouse pluripotent stem cell differentiation, we defined the temporal remodelling of the chromatin landscape mediated by Mesp1. We identified different enhancers that are temporally regulated to erase the pluripotent state and specify the pools of CPs that mediate heart development. We found that Mesp1 acts as a pioneer transcription factor (TF) and identified Zic TFs as essential cofactors that regulate Mesp1 pioneer activity at key mesodermal enhancers, thereby regulating the chromatin remodelling and gene expression associated with specification of the different populations of CPs in vivo. Our study identifies the dynamics of the chromatin landscape and enhancer remodelling associated with temporal patterning of early mesodermal cells into the distinct populations of CPs that mediate heart development.

Funding Source: B.S. is supported by an Aspirant FNRS Fellowship

Keywords: Gene expression dynamics, Cardiovascular progenitor specification, Mesp1 gene regulatory network

Poster: 602

SPECIES-SPECIFIC OCT4 UPSTREAM REGULATORY REGION-BASED REPORTER SYSTEM FOR MONITORING PLURIPOTENCY IN PORCINE EMBRYONIC STEM CELLS

Lee, Chang-Kyu[1], Kim, Seung-Hun[2], Choi, Kwang-Hwan[2], Lee, Mingyun[2], Jeong, Jinsol[2], Lee, Dong-Kyung[2], Oh, Jong-Nam[2], Choe, Gyungcheol[2], Lee, Chang-Kyu[2]

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The present study examined the function of porcine OCT4 (POU5F1) enhancers in porcine embryonic stem cells. OCT4 is one of the master regulators for the pluripotency of early mammalian embryonic development and embryonic stem cells. It has two regulatory elements: distal enhancer (DE) and proximal enhancer (PE). These are located in the OCT4 upstream regulatory region. Two enhancers activated under different conditions such as naïve and primed. It is known that two enhancers are activated to produce OCT4 simultaneously or sequentially depending on the state of pluripotency. Due to the importance of the OCT4 upstream region-based reporter system in porcine-specific pluripotent studies, many porcine OCT4 reporter systems were reported. However, the porcine-specific OCT4 reporter system has never been transfected in porcine embryonic stem cells, nor has its function been identified. We performed functional tests of the previously established porcine-specific OCT4 reporter system in the porcine embryonic stem cells. Porcine embryos were micro-injected with the pOCT4-ΔPE-eGFP (DE-GFP) containing a distal enhancer and core promoter and pOCT4-ΔDE-DsRed2 (PE-RFP) containing a proximal enhancer and core promoter. Embryonic stem cells were established with the blastocysts with a porcine OCT4 reporter system. We analyzed mRNA and protein expressions of GFP and RFP using quantitative real-time polymerase chain reaction and confocal microscopy. The introduced reporter system could work in pluripotent cells and the expression of GFP and RFP was observed simultaneously in the embryonic stem cells. Comparing the expression levels of distal and proximal enhancers with other naïve and primed state pluripotent cells, the porcine embryonic stem cells were close to a primed state, not a naïve state. The reporter system was disabled after the differentiation of embryonic stem cells. These results showed that the porcine OCT4 reporter system which enables the non-destructive analysis in porcine embryonic stem cells and it could be applied to study species-specific pluripotency and to help the establishment of naïve embryonic stem cells in the pig.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT; 20012411), and the National Research Foundation of Korea (NRF) grant (2021R1A2C4001837).

Keywords: Reporter system, OCT4, Embryonic stem cells

Poster: 603

BMP4 AND SECRETED NOGGIN FORM SPATIAL DIFFERENTIATION PATTERN OF HUMAN IPS CELLS

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We previously reported that BMP4 effects on cell differentiation were examined using a one-directional perfusion culture chamber and human induced pluripotent stem (iPS) cells. As a result, we found that cells located upstream of the chamber differentiated, whereas downstream cells remained undifferentiated. Because Noggin specifically binds to BMP4 to prevent BMP4 from binding to receptors, we hypothesized that cell differentiation patterning can be attributed to secreted Noggin from cells to inhibit differentiation. We simulated substance concentrations in a reaction-diffusion model to test our hypothesis. The experimental results were reproduced in the simulation by two conditions in which cells gradually differentiated and secreted Noggin depending on BMP4 exposure, and in which cells constantly secrete Noggin independently of BMP4 exposure, suggesting that our hypothesis is appropriate. Thus, cell differentiation patterning interaction mediated by BMP4 and Noggin can be controlled in vitro as with in vivo.

Keywords: microfluidics, differentiation-inducing factor, morphogen

Poster: 604

PROGRESSIVE SPATIAL SELF-ORGANIZATION OF PLURIPOTENT STEM CELL IN COLONIES AND AGGREGATES

Elsafi Mabrouk, Mohamed H.[1], Goetzke, Roman[2], Abgnale, Guilio[3], Yesilyurt, Burcu[4], Salz, Lucia[2], Zeevaert, Kira[2], Ma, Zhiyao[2], Szymanski, Marcelo[5], Pachauri, Vivek[6], Schnakenberg, Uwe[6], Li, Ronghui[7], Costa, Ivan G.[7], Zenke, Martin[8], Wagner, Wolfgang[2]

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Pluripotent stem cells (PSCs) comprise heterogeneous subpopulations, even under pluripotent growth conditions. The highest up-regulation of pluripotency markers is observed at the rim of PSC colonies. In this study, we have further analyzed the development of such spatial heterogeneity within colonies and early 3D aggregates of induced pluripotent stem cells (iPSCs). To this end, we utilized two approaches of geometric confinement of iPSCs: cells were either cultured on PDMS pillars, or micro-contact printed (µCP) substrates to define size and shape of iPSCs colonies. Immunofluorescence analysis revealed that pluripotency markers, such as OCT4, E-cadherin, and NANOG, became within six days progressively up-regulated at the edge of the colonies, irrespective of size and shape of the colonies. Single-cell RNA-sequencing (scRNA-Seq) and spatial reconstruction of gene expression demonstrated that the POU5F1 (OCT4)-high subpopulations, residing at the edge of the colony, have distinct gene expression profiles. Gene Set Enrichment Analysis (GSEA) revealed prominent up-regulation of TGF-ß pathway including NODAL and its inhibitor LEFTY. Immunofluorescence showed that NODAL follows the same dynamic of OCT4 organization. Notably, disruption of calcium-dependent cell junctions or inhibition of the TGF-ß pathway was associated with the loss of self-organization of the colonies. Interestingly, after 5 to 7 days, the iPSC colonies detached spontaneously from micro-contact printed substrates to form 3D aggregates. This new method allowed generation of embryoid bodies (EBs) of controlled size, without any enzymatic or mechanical treatment. Subsequently, we analyzed how the spatial self-organization is developing in early three dimensional (3D) aggregates of iPSCs. We found that OCT4 and NODAL were also higher expressed at the outer 3D sphere and this was also reflected by the differential gene expression of OCT4-high versus OCT4-low subpopulations in scRNA-seq data. Taken together, our results provide additional insight into heterogeneity and self-organization of pluripotent stem cell colonies in their transition to embryoid bodies.

Keywords: Stem cell heterogeneity, self-organization, single cell RNA-Sequencing

Poster: 605

YAP KNOCKOUT SUPRESSES EARLY GERM LAYER SPECIFICATION OF HUMAN IPSC-DERIVED EMBRYOID BODIES

Zeevaert, Kira, Maaßen, Catharina, Elsafi Mabrouk, Mohamed Hamdy, Schmidt, Marco, Wagner, Wolfgang, Goetzke, Roman

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Induced pluripotent stem cells (iPSCs) form aggregates that recapitulate aspects of the self-organization in early embryogenesis. Within few days, the cells undergo transition from epithelial-like structures to organized three-dimensional embryoid bodies (EBs) with upregulation of specific genes for mesodermal, endodermal, and ectodermal differentiation. The Yes-associated protein (YAP) is a downstream effector of the Hippo pathway and an essential mechanotransducer, which is suggested to affect growth of embryonic structures. To further investigate the relevance of YAP for early differentiation in EBs, we used CRISPR/Cas9 technology to generate homozygous YAP knockout (YAP-/-) iPSCs. YAP deficiency was confirmed by Western Blot and immunophenotypic analysis. These knockout cells revealed typical iPSC morphology, maintained expression of the pluripotency markers E-cadherin and OCT-

4, and a positive epigenetic Epi-Pluri-Score. Subsequently, we analyzed the differentiation capacity of wildtype and YAP-/- iPSCs towards the three germ layers in two-dimensional media-directed differentiation assays. Immunofluorescence and qRT-PCR revealed significantly lower expression of Brachyury and PAX6 upon differentiation toward mesoderm and ectoderm, respectively. Furthermore, DNA methylation profiles of undifferentiated YAP-/- iPSCs indicated an inhibited germ layer specification compared to wildtype iPSCs and demonstrated significant epigenetic differences between wildtype and YAP-/- iPSCs after germ layer formation. Notably, a complete loss of mesodermal, endodermal, and ectodermal differentiation was observed in three-dimensional EBs that were generated from YAP-/- iPSCs. In contrast, EBs of wildtype controls comprised subpopulations with clear up-regulation of Brachyury (mesoderm), PAX6 (ectoderm), and GATA6 (endoderm). These results indicate that YAP is relevant for early germ layer specification of iPSCs, particularly during self-organization and differentiation of EBs.

Keywords: Embryoid body, Germ layer specification, YAP

Poster: 606

INVESTIGATING THE ROLE OF DUPLICATED PSEUDOGENES IN HUMAN PLURIPOTENCY

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Gene duplication events play an important role in genome evolution. They can also create developmental strategies that differ between species, however, the regulation and functional contribution of duplicated genes in human early development and pluripotency is poorly understood. In this study, we have investigated a duplicated pseudogene of a key transcription factor in human pluripotent stem cells (hPSCs). Using evolutionary genomic analysis we found that the duplication event occurred in a common ancestor of hominoids and Old World monkeys, but an intact copy has been retained only in hominoids. In the human genome, we found a series of non-synonymous mutations and deletions that distinguish the gene duplicate from the ancestral copy and identified its potential isoforms. By examining RNA-seq datasets, we uncovered the expression pattern of the duplicate gene and found that it differed from its ancestral copy. The duplicated gene is highly expressed in naïve hPSCs only, whereas the ancestral copy is highly expressed in both naïve and primed hPSCs. We also identified that the duplicated gene is expressed in the human preimplantation embryo similarly to its ancestral copy, however, the expression patterns start to diverge as the embryo progresses in development. We sought to understand whether this gene duplication event had resulted in paralogue neofunctionalisation and/or potential dominant negative function over the ancestral copy, or whether they had evolved to share the same role. This was tested using overexpression and knockdown experiments in primed and naïve hPSCs. While no full dominant negative effect was detected, a partially differentiated cell phenotype was observed when the duplicated gene was overexpressed. Over a short time-course, knockdown of the ancestral gene resulted in cell differentiation, whereas disrupting the expression of the duplicated gene did not cause the same effect. We are currently investigating the long-term loss of function effect, as well as attempting to epitope tag the duplicated gene for further functional analysis. Overall, this study has focused on a gene duplication event that has predicted functional consequences in hPSCs, exemplifying a case of potential gene subfunctionalisation with relevance to human developmental and stem cell biology.

Funding Source: This work is funded by the BBSRC, MRC, Darwin Trust of Edinburgh and Cambridge Trust.

Keywords: gene duplication, pluripotency, naïve pluripotent stem cells

Poster: 607

LINEAGE PRIMING BY DPPA2/4 IN HUMAN PLURIPOTENT STEM CELLS

Malcolm, Andrew A.[1], Bendall, Adam[1], Collier, Amanda J.[2], Bevan, Stephen[1], Fabian, Charlene[3], Semprich, Claudia[1], Reik, Wolf[1]

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Precise co-ordination of cell fate specification during human early development is a vital yet poorly understood process. Understanding how cells navigate the dynamic transcriptional and epigenetic changes associated with germ layer allocation is critical to identify how to improve the derivation of functional cell types, and also to understand why embryogenesis fails at this gastrulation stage in a large proportion of pregnancies. Epigenetic priming has been proposed to facilitate robust and appropriate differentiation through establishment of a competent but repressed chromatin landscape, typically through the co-existence of both active-associated and repression-associated histone modifications at the same promoter or enhancer. The pluripotency-associated factors DPPA2 and DPPA4 have been proposed to target this chromatin landscape in mouse embryonic stem cells but their role in the human context has not been well studied. During human embryo development, these proteins are expressed from the 8-cell stage until late gastrulation and are thereby well-positioned to regulate cell fate specification during germ layer segregation. Through CRISPR-Cas9-mediated genetic knock-out and subsequent cellular and molecular phenotyping in primed human pluripotent stem cells (hPSCs), we describe here new roles for DPPA2 and DPPA4 in regulating lineage gene expression and cell fate specification in human. Depletion of these factors in hPSCs leads to aberrant deregulation of developmental transcriptional programs. Failure to constrain expression of developmentally critical genes results in DPPA2-and DPPA4-deficient hPSCs unable to fine-tune appropriate transcriptional responses to signalling factors, leading to abnormal cell differentiation. We find that DPPA2 and DPPA4 are enriched at bivalent promoters and poised enhancers, suggesting the developmental defects are potentially due to changes in histone modifications at these loci, thus placing DPPA2/4 as putative regulators required to maintain the `poised` epigenome. Unravelling the mechanisms that underpin epigenome priming will provide valuable insights into how cells control gene regulatory responses to acquire appropriate cell fates in a spatially and temporally regulated environment, such as the developing human embryo.

Funding Source: Andrew Malcolm is the recipient of a Wellcome Trust four-year PhD studentship.

Keywords: Epigenetics, Pluripotency, Differentiation

Poster: 608

MENSTRUAL BLOOD DERIVED STROMAL CELL ANALYSIS REVEALS SOX15 TRIGGERS OOCYTE-BASED CELL REPROGRAMMING TO A DISTINCTIVE HUMAN PLURIPOTENT STATE

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Our long term goal is to provide new insights into cellular reprogramming by analyzing molecular pathways, and identifying new factor and mechanisms, that could play an important role in cell reprogramming. Our starting hypothesis propose that oocyte reprogramming factors (ORP) bear responsibility for the exceptional reprogramming capacity of somatice cell nuclear transfer (SCNT) and that studying the genes and gene products present in the oocyte (more specifically the unfertilized metaphase II oocyte) can help us understand how pluripotency is acquired in somatic cells and define different pluripotent states within reprogrammed cells that can more closely resemble the natural progression after fertilization (and thus improve their application). Our results show that combination of oocyte-enriched genes generate efficient reprogramming that follows distinguishable reprogramming mechanisms and generates iPSCs with a distinctive pluripotent state with relevant functional consequences, showing increased differentiation potential. We analyze the molecular profile of different human somatic cell types. We show menstrual blood derived stromal cells (MnSCs) have a distinct and reprogramming-prone profile and we identify SOX15 from their oocyte-related signature as a prominent responsible candidate. SOX15 orchestrates an efficient oocyte-based reprogramming combination when overexpressed with the also oocyte-enriched histone chaperone ASF1A and OCT4, and through specific mechanism, generates primed iPSCs with distinguishable pluripotent state that further present higher differentiation capacity than canonical primed iPSCs. We have also explored the generation of naïve pluripotent oocyte-based iPSC to define their pluripotent state.

Funding Source: Ministerio de Economía y Competitividad Gobierno de España (RYC-2014-15410 and SAF2015-66105-R), Consejería Economía y Conocimiento Junta de Andalucía-FEDER (UMA18-FEDERJA-107)

Keywords: iPSCs, reprogramming, Oocyte, pluripotency, Menstrual blood derived stromal cells

Poster: 609

UNDERSTANDING THE IDENTITY AND ORIGIN OF THE HUMAN AMNIOTIC FLUID STEM CELLS DURING FETAL DEVELOPMENT

Gerli, Mattia F.[1], Beesley, Max A.[1], Pellegrini, Marco[1], Martini, Paolo[2], Panariello, Francesco[3], Kunpalin, Yada[1], Subramaniam, Sindhu[1], Cacchiarelli, Davide[3], Romualdi, Chiara[2], David, Anna[4], De Coppi, Paolo[1]

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The amniotic fluid (AF) surrounds and protects the fetus during development, providing trophic and mechanical support to its growth. This fluid contains a variety of cells shedding from embryonic and extra-embryonic tissues, long utilised for prenatal diagnosis. Importantly, a population of mesenchymal cells with broad multi-lineage differentiation ability, can be consistently isolated from the AF. The so named Amniotic Fluid Stem Cells (AFSCs) can be expanded and differentiated during gestation, making them an ideal candidate for fetal and neonatal autologous regenerative medicine. In the last decade, numerous studies focused on investigating the therapeutic relevance of the AFSCs for various tissue compartments, such as for the treatment of skeletal muscle, cardiovascular, respiratory, hepatic and hematopoietic conditions. However, very little is yet known about the AFSCs origin, lineage identity or role during human development. While GMP methods to isolate and culture these cells have been developed, the lack of knowledge about AFSCs biology has relevant impact on these cells' potential clinical translation. With the aim of addressing these questions, we isolated primary mesenchymal stem cells from a broad library of human fetal and extraembryonic tissues, which are exposed or actively contributing to the AF at different gestational stages. We then compared these cells' RNA sequencing profile, with the one of 18 AFSCs lines isolated from therapeutic amniocenteses at different post-conceptional weeks. Thanks to our analysis, we have identified the fetal tissue from which AFSCs originate, highlighting the mechanism driving this process. Moreover, we have developed a novel cell sorting strategy - including novel specific surface markers - that significantly increase efficiency and speed of the AFSCs isolation procedure. We have validated our results using an organoid model mimicking the AFSC generation process in vitro. Finally, we have used a combination of bulk and single cell sequencing datasets, to identify the specific target cell subpopulation within the tissue from where the AFSCs originate. Overall, our results clarify identity and origin of AFSCs, paving the way for novel regenerative medicine approaches based on these cells.

Funding Source: This work is funded by a European Commission H2020 Marie Skłodowska-Curie Action fellowship, by the NIHR Great Ormond Street Biomedical Research Centre, the UCL GOS Institute of Child Health and UCL Global Engagement Support Funds

Keywords: Amniotic fluid stem cells, Fetal development, Mesenchymal stem cells

Poster: 610

RAPID AND CONSISTENT CLASSIFICATION OF KIDNEY ORGANOID SINGLE CELL RNA-SEQ DATASETS USING DEVKIDCC

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While single cell transcriptional profiling has greatly increased our capacity to interrogate biology, accurate cell classification within and between datasets is a key challenge. This is particularly so in pluripotent stem cell-derived organoids which represent a model of a developmental system. Here, clustering algorithms and selected marker genes can fail to accurately classify cellular identity while variation in analyses makes it difficult to meaningfully compare datasets. Kidney organoids provide a valuable resource to understand kidney development and disease. However, direct comparison of relative cellular composition between protocols has proved challenging. Hence, an unbiased approach for classifying cell identity is required. The R package, scPred, was trained on multiple single cell RNA-seq datasets of human fetal kidney. A hierarchical model classified cellular subtypes into nephron, stroma and ureteric epithelial elements. This model, provided in the R package DevKidCC (github.com/KidneyRegeneration/DevKidCC), was then used to predict relative cell identity within published kidney organoid datasets generated using distinct cell lines and differentiation protocols, interrogating the impact of such variations. DevKidCC was used to directly compare between distinct kidney organoid protocols, identifying differences in relative proportions of cell types and highlighting variations in stromal and unassigned cell types, nephron progenitor prevalence and relative maturation of individual epithelial segments. Of note, DevKidCC was able to distinguish distal nephron and ureteric epithelium, cell types with overlapping profiles that have previously confounded analyses. When applied to a variation in protocol via the addition of retinoic acid, DevKidCC identified a consequential depletion of nephron progenitors accompanied by increased proximal tubule prevalence and improved parietal epithelial cell patterning. The application of the tool to all available datasets is summarised in an interactive Shiny application (sbwilson91.shinyapps.io/devkidcc_interactive/). This tool will enable the consistent and rapid comparison of kidney organoid protocols, driving improvements in patterning to kidney endpoints and validating new approaches.

Keywords: cell identity prediction, human developing kidney, kidney organoid

Poster: 611

HIGH THROUGHPUT 3D AIRWAY ORGANOID MODEL GENERATION TO ASSESS INFECTIVITY OF ENMERGING VIRUES

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Organoids are organ-like tissues derived from pluripotent stem cells or isolated organ progenitor cells. The cells self organize into the differentiated cell types that are present in the organ of interest and recapitulate the structural and functional characteristics of the native tissue. These 3D complex structures provide a unique opportunity to model human organ development in an in vitro culture system similar to in vivo conditions. Organoids therefore have the potential to be used for organ replacement, disease modeling, and drug testing, In terms of virology, airway organoids have been derived from a variety of origin cell types including trachea, large airway basal, alveolar, human induced pluripotent stem cells (hiPSC), and embryonic lung4. These 3D structures can recapitulate the physiochemical and functional properties of the differentiated airway epithelium containing ciliated, goblet, and basal cells, and are effective tools for respiratory virus research and disease modeling. In this study, we investigated the suitability of Matrigel matrix-3D plates as a 3D virology experimental platform for high throughput generation of airway organoids. In this model, airway organoids demonstrated differentiated gene expression, expressed proximal epithelial ciliated, goblet and basal cells, and virus host receptors ACE2 and a2-6-linked sialic acids .Corning Matrigel matrix 3D-plates can therefore be used for high throughput study of human respiratory virus infectivity including SARS-CoV-2 and influenza virus.

Keywords: High Throughput, Airway Organoid, influenza virus

Poster: 612

REPROGRAMMING OF HUMAN FIBROBLASTS TO PLURIPOTENCY INDUCES CENP-A CHROMATIN DEPLETION

Pereira, Carolina[1], Milagre, Ines[1], Pereira, Carolina[1], Oliveira, Raquel A.[1], Jansen, Lars E.[2]

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Pluripotent stem cells (PSCs) are central to development as they are the precursors of all cell types in the embryo. Therefore, maintaining a stable karyotype is essential, both for their physiological role as well as for their use in regenerative medicine. Karyotype abnormalities in PSCs in culture are common but the underlying causes remain unknown. To gain insight, we explore the composition of the centromere and kinetochore in human embryonic and induced PSCs. Centromere function depends on CENP-A nucleosome-defined chromatin. We show that while PSCs maintain abundant pools of CENP-A, CENP-C and CENP-T, these essential centromere components are strongly reduced at stem cell centromeres. Outer kinetochore recruitment is also impaired to a lesser extent, indicating an overall weaker kinetochore while the inner centromere protein Aurora B remains unaffected. We further show that, similar to differentiated human cells, CENP-A chromatin assembly in PSCs requires transition into G1 phase. Finally, reprogramming experiments indicate that reduction of centromeric CENP-A levels is an early event during dedifferentiation, coinciding with global chromatin remodelling. Our characterization of centromeres in human stem cells suggests a possible link between impaired centromere function and stem cell aneuploidies.

Funding Source: Fundação para a Ciência e Tecnologia project: PTDC/BIA-CEL/31502/2017, EU's Horizon 2020 MCSA grant no. 704763 to I.M., ERC-2013-CoG-615638 and SRF in Basic Biomedical Science to L.E.T.J. and EMBO Installation Grant IG2778 to R.A.O.

Keywords: Centromere, Chromatin, CENP-A

Poster: 614

THE GCNF-BCL11A CONNECTION AS A REGULATOR OF HUMAN NEURAL STEM CELL DIFFERENTIATION

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A number of experimental observations suggest that the orphan nuclear factor and transcriptional repressor GCNF/NR6A1 plays a critical role in neural lineage development. For example, GCNF has been reported to be required for the development of definitive neural stem cells, neural tube closure and correct regionalization. We set out to study the role of GCNF in neuronal differentiation of human pluripotent stem cell-derived neural stem cells (hNSCs). GCNF is expressed in hNSCs with levels decreasing upon and across neuronal differentiation. Overexpression of GCNF inhibits neuronal differentiation and leads to the formation of dense cell clusters consisting of proliferative neural stem cells. Vice versa, inversion of the repressive effect of GCNF with a GCNF-VP16 fusion construct accelerates neuronal differentiation remarkably. The effect was comparable to that of forced NGN2 expression, an established paradigm for forward programming of pluripotent stem cells into neurons. However, in contrast to sporadic and NGN2-mediated neuronal differentiation, GCNF-VP16 overexpression leads to the formation of highly uniform neuronal networks without typical neuronal cluster formation. Transcriptome analyses and in silico target prediction depicted BCL11A as a prominent putative downstream effector of GCNF. Interestingly, GCNF and BCL11A expression exhibit opposite dynamics during NSC differentiation with BCL11A levels increasing alongside neuronal differentiation. Binding of GCNF to the BCL11A promoter was confirmed by ChIP studies. In addition, overexpression of GCNF in differentiated NSCs decreased BCL11A transcript levels, whereas GCNF-VP16 overexpression resulted in a pronounced increase in BCL11A. Our data depict GCNF as an important modulator of human neurogenesis and suggest that this effect is mediated via BCL11A.

Keywords: GCNF, transcriptional repressor, neurogenesis

Poster: 615

POTENTIAL OF HUMAN AMNIOTIC EPITHELIAL CELLS FOR SKIN RECONSTITUTION AND ITS REPROGARMMING

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Although tissue engineered skin substitutes provide a new therapy for treatment of acute and chronic skin wounds, it is limited by the availability of suitable source of cells. Amniotic epithelial cells (AECs) have been used in 3D skin reconstitution instead of keratinocytes (KCs); however, there are still great gaps. To better understand the commonalities and differences between human AECs and KCs, we compared their biological features and functions and found that AECs expressed comparable levels of undifferentiated markers of KCs and they phagocytosed melanosome provided by human melanocytes in both in vitro and ex vivo systems. Transcriptome analysis showed their highly similarities, including epidermis development, morphogenesis of an epithelium, regulation of epidermis development, cell junction organization, molting cycle and hair cycle. However, significant differences still existed. Overexpression of TP63 and activation of NOTCH pathway helped to short their gaps and reprogram AECs into KCs. In view of the above results and the multiple advantages of AECs in the field of regeneration medicine, AECs are expected to be a good candidate for skin reconstitution in the future.

Funding Source: National Natural Science Foundation of China (82070638 and 81770621); Natural Science Foundation of Jiangsu Province (BK20180281).

Keywords: Amniotic epithelial cells, Keratinocytes, Skin reconstruction

Poster: 616

AN INTEGRATED ATLAS OF HUMAN PLACENTAL DEVELOPMENT DELINEATES LINEAGE IDENTITY AND ESSENTIAL REGULATORS OF TROPHOBLAST STEM CELLS

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The trophoblast lineage safeguards fetal development by mediating embryo implantation, immune-tolerance, nutritional supply and gas exchange. However, the formation of the placenta from trophoblast remains poorly understood. Human trophoblast stem cells (TSCs) provide a platform to delineate lineage specification of placental tissues, yet, the precise in vivo counterpart of TSCs has remained elusive. To address these issues, we integrated six single-cell transcriptome datasets into a unified atlas of human trophoblast development from zygote to mid-gestation and performed single-cell profiling of TSCs in Okae and Turco culture conditions. We demarcate the transcriptional networks of trophoblast lineages in vivo and construct a probabilistic model of trophoblast cell fate acquisition, which indicates a role for MAPK signalling in trophoblast differentiation. Conventional and probabilistic algorithms consistently mapped TSCs in close proximity to the early cytotrophoblast, with Okae cells leaning towards preimplantation trophoblast. We functionally assess the cytotrophoblast gene regulatory network in vitro and demonstrate that MAPK inhibition accelerates trophoblast differentiation. Thus, our atlas of human placental development provides a powerful analytical resource to delineate trophoblast lineage specification and to gauge the developmental stage of in vitro cultured cells.

Keywords: trophoblast stem cells, placental development, transcriptome atlas for trophoblast development

00:00 - 1:00 EDT

POSTER SESSION 6

MODELING DEVELOPMENT AND DISEASE

Poster: 625

CARDIAC-SPECIFIC SPLICING FACTOR MODULATES ORGANIZATION OF SARCOMERE STRUCTURE IN HUMAN EMBRYONIC STEM CELL DERIVED-CARDIOMYOCYTES

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RNA splicing factors generate different protein variants from a single gene, thereby increasing proteomic diversity and participating in the developmental process, cell specification and human diseases. Impaired alternative spicing leads to developmental defects and many diseases including dilated cardiomyopathy (DCM), a major cause of heart failure. RNA-binding motif protein 24 (RBM24) is a cardiac-specific splicing factor. Previous studies have shown that RBM24 is essential for cardiogenesis and DCM pathogenesis in animal models. However, the underlying molecular mechanism has not yet to be elucidated. Notably, at present RBM24 mutation has not been identified in DCM patients or patients with heart diseases. Therefore, it is compelling needed to understand the role of RBM24 in human cardiac development and cardiomyopathy. The sources of human materials are always a bottleneck for studying the function of human genes. Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the capacity to differentiate into all cell types (e.g. cardiomyocytes). Therefore, hPSCs can provide unlimited starting materials for studying human cardiogenesis and cardiomyopathy. Hence, in this study, we carry out comprehensive approaches to elucidate the role of RBM24 in cardiac cell development and cardiomyopathy using hESC as a model. We first showed that RBM24 ablated cardiomyocytes (CMs) exhibited defects in cardiac cell development, including sarcomere disorganization, abnormal calcium handling ability, and reduced proliferation. Moreover, impairment of the RBM24-mediated RNA splicing process therefore could cause DCM. Furthermore, alternative splicing assay revealed that RBM24 could regulate alternative splicing of multiple genes, disrupting sarcomere structure. In conclusion, our preliminary studies revealed that RBM24 plays an essential role in human sarcomere formation and may also be involved in DCM pathogenesis.

Keywords: Cardiac-specific splicing factor, Cardiac cell development, Human pluripotent stem cells

Poster: 626

HIGH CELL DENSITY PROMOTED CARDIAC DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS INHIBITING WNT SIGNALING SUPPLIED FROM AUTO/PARACRINE FACTORS

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) have received increasing attention for their clinical use. Many protocols induce cardiomyocytes at an initial high cell density (100% confluence) to utilize cell density effects as hidden factors for cardiomyocyte differentiation. Previously, we established a protocol to induce hiPSC differentiation into cardiomyocytes using a defined culture medium and an initial low cell density (1% confluence) to minimize the hidden factors. Here, we investigated the key factors promoting cardiomyocyte differentiation at an initial low cell density to clarify the effects of cell density. Co-culture of hiPSCs at an initial low cell density with those at an initial high cell density showed that signals secreted from cells (auto/paracrine factors) and not cell-cell contact signals, played an important role in cardiomyocyte differentiation. Moreover, although cultures with initial low cell density showed higher expression of anti-cardiac mesoderm genes, earlier treatment with a Wnt production inhibitor efficiently suppressed the anti-cardiac mesoderm gene expression and promoted cardiomyocyte differentiation by up to 80% at an initial low cell density. These results suggest that the main effect of cell density on cardiomyocyte differentiation is inhibition of Wnt signaling at the early stage of induction, through auto/paracrine factors.

Funding Source: This work was supported by a grant AMED (JP17bk0104011h0005)

Keywords: cardiomyocyte, human induced pluripotent stem cell, cell density

Poster: 627

GENETIC AND PHARMACOLOGICAL STUDIES IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES REVEALS ACID SENSING ION CHANNEL 1A AS A NOVEL THERAPEUTIC TARGET AGAINST CARDIAC ISCHEMIA-REPERFUSION INJURY

Redd, Meredith A.[1], Scheuer, Sarah E.[2], Saez, Natalie J.[1], Yoshikawa, Yusuke[3], Chiu, Han S.[1], Cuellar-Partida, Gabriel[4], Peart, Jason N.[5], See Hoe, Louise E.[6], Chen, Xiaoli[1], Sun, Yuliangzi[1], Suen, Jacky Y.[7], Maljevic, Snezana[7], Quaife-Ryan, Gregory A.[8], Hudson, James E.[8], Porrello, Enzo R.[9], White, Melanie Y.[10], Cordwell, Stuart J.[10], Fraser, John F.[6], Petrou, Steven[7], Reichelt, Melissa E.[3], Thomas, Walter G.[3], King, Glenn F.[1], Macdonald, Peter S.[2], Palpant, Nathan J[1]

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Ischemia-reperfusion injury (IRI) results in myocardial cell death and compromised cardiac function making it a leading risk factor for heart failure. We used UK Biobank data to identify human genetic loci associated with cerebrovascular ischemic injuries and revealed the acid sensing ion channel 1a (ASIC1a) associated with major coronary heart disease and myocardial infarction. Using human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) in vitro, we demonstrate that genetic ablation of ASIC1a improves cardiomyocyte viability after acute IRI. Furthermore, pharmacological activation of the channel using snake venom MitTx potentiates cardiac cell death in wildtype but not in ASIC1a KO hPSC-CMs thereby identifying ASIC1a is a novel therapeutic target to improve the tolerance of cardiac tissue to IRI. We therefore tested therapeutic blockade of ASIC1a using a specific and potent pharmacological ASIC1a inhibitor, Hi1a, derived from venom of the Australian funnel web spider. Using hPSC-CMs we show that acute addition of Hi1a to hPSC-CMs has no impact on normal cardiac electromechanical coupling but significantly improves survival after in vitro IRI. Patch clamp electrophysiological assays on all major cardiac ion channels further validated no off-target toxicity of Hi1a. To test the role of ASIC1a in whole organ physiology, we demonstrate potent therapeutic benefit from genetic ablation or pharmacological inhibition of ASIC1a in a murine model of whole organ ex vivo IRI. To test the potential clinical utility of ASIC1a inhibitors, we used an in vivo model of myocardial infarction and two models of ex vivo heart transplant to show that ASIC1a inhibition improves post-IRI cardiac viability and function. Collectively, our data provide compelling evidence for a novel pharmacological strategy involving ASIC1a blockade as a novel cardioprotective therapy to improve the viability of hearts subjected to IRI.

Keywords: myocardial ischemia-reperfusion injury, acidosis, cardioprotection

Poster: 628

DRUG REPURPOSING FOR SARS-COV-2 INFECTION VIA ACE2 INTERNALIZATION IN IPSC CARDIOMYOCYTES

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A new pandemic of pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged and its severe and prognostic symptoms have become a social issue. Especially, SARS-CoV-2 has been reported to damage heart, lung and brain, which increases the risk of long-term health problems. SARS-CoV-2 infection is mainly initiated by binding of its surface Spike protein to angiotensin converting enzyme 2 (ACE2) on the host cell membrane followed by internalization via endocytosis-mediated pathway. Repurposing existing drugs is a rapid and effective way to provide new treatments by identifying new indications from existing approved drugs that have enough pharmacological and safety profiles. In the study, we evaluated the inhibitory effects of approved drugs on Spike protein-induced ACE2-GFP internalization in HEK293 cells. We found a compound which prevent SARS-CoV-2-induced infection and proliferation in TMPRSS2-expressing VeroE6 cells. In addition, the compound inhibited RNA replication in human iPSC-derived cardiomyocytes. These results provide a new concept that the inhibition of ACE2 internalization is a novel strategy to provide COVID-19 treatment and prevent heart damage.

Funding Source: AMED

Keywords: SARS-CoV-2, Drug Repurposing, iPSC-cardiomyocytes

Poster: 629

UNRAVELING THE PATH OF EPICARDIAL CELL SPECIFICATION FROM HUMAN PLURIPOTENT STEM CELLS IN 3D CONDITIONS

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The epicardium is the epithelial layer that covers the outer surface of the heart, playing an essential role in normal human heart development. Although it is known that the epicardial cells originate outside the primitive heart tube in a transient structure known as pro-epicardium, its embryonic origin remains unclear, especially in humans. In the present work, it was developed a novel 3D platform to generate pro-epicardial cells (PECs) from human pluripotent stem cells (hPSCs) with the aim of improving the knowledge about the origin of the progenitor cell population from where PECs are specified and to perform a deeper molecular characterization of these cells. Since it has been suggested in the literature that both CMs and PECs are generated from lateral plate/splanchnic mesoderm progenitors (LPM), we decided to take advantage of our previously reported 3D CM differentiation protocol, and adapt it for PECs commitment.

For that purpose, we explored the impact of activating the signaling pathways Wnt, BMP4 and RA. These studies revealed that Wnt, and at lower extent RA, signaling pathways are the most critical stimuli to allow the successful generation of WT1+-PECs and completely abolish CMs commitment. In addition to that, and since it was observed that the progenitor cell pool from where PECs and CMs were previously induced is an heterogenous cell population, we decided to optimize the first steps of hPSCs commitment into pre-cardiac progenitor cells in order to maximize PECs generation. It was observed that the progenitor cell population that potentiates PECs commitment is distinct from the one that allows CM differentiation, indicating developmental differences between both cell types. We are now performing a deeper characterization of these two populations by transcriptomic analysis. In conclusion, we established an efficient protocol that allows the generation of more than 90% of WT1+-PECs after 11 days of differentiation, in a 3D environment, which is now being used to study the developmental origin of PECs and to understand the point of divergence in relation to CM commitment.

Keywords: human pluripotent stem cell-derived pro-epicardial cells, 3D differentiation platform, mesoderm progenitors of pro-epicardial cells

Poster: 632

ANALYSIS OF LHX1 GENE REGULATORY NETWORK REVEALS A KEY ROLE FOR KCTD1 IN MOUSE EMBRYONIC HEAD FORMATION

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Embryonic development is driven by a series of molecular instructions encoded by the transcription factors (TFs) that drive the formation of the body plan and the specialisation of tissue precursor cells. Analysis of gastrulating mouse embryos has demonstrated that key TFs such as LIM homeobox 1 (Lhx1) are indispensable for head and face development. The aim of this project is to identify and functionally characterise the genetic targets of LHX1 to refine the gene regulatory network for embryonic head formation. We have conducted RNA-sequencing, ATAC-sequencing and used DamID-seq on novel mouse embryonic stem cell derived organoids as well as gastrulating mouse embryos to identify genomic regions that are directly regulated by LHX1. The integrated data revealed a role for LHX1 in regulating the Wnt and hedgehog signalling pathways in the anterior embryo. We identified the Wnt-antagonist gene Kctd1 as a direct genetic target of LHX1. Using Kctd1-/- embryoid bodies, we have shown Kctd1 plays a key role in establishing the anterior mesendodermal cell population (a precursor lineage to the embryonic head). Additionally we found that Kctd1 is essential for driving the differentiation of neural tissue in mouse embryos using Kctd1-/- stem cell derived chimera embryos. By determining novel gene regulators in the LHX1 network, we gained a valuable insight into the cellular and molecular mechanisms building the body plan in the early mammalian embryo.

Keywords: gene regulatory network, head development, wnt signalling

Poster: 633

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

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In-vivo embryogenesis demonstrates a flawless example of a self-organized system that uses a combination of diffusible morphogens and the effect of physical stress of the endometrial constraint as guidance for germ layer differentiation and their organization. Modulating various physical environmental factors has supported the growth and development of a pre-gastrulation mouse embryo in in-vitro studies. Although a lot of information is available on how diffusible morphogens affect gene expression and signaling dynamics for germ layer differentiation, the mechanistic role in inducing these events is poorly understood. Due to the ethical and physiological limitations of studying human embryos, in-vitro models present a better platform to understand the contribution of mechanical stress towards the differentiation and self-organization capacity of pluripotent stem cells. Previous studies have demonstrated the role of colony geometry in germ layer organization of embryonic stem cells in response to exogenous BMP4 induction. Here, we present an in-vitro gastrulation model, which uses polyacrylamide hydrogels as culture substrates to present physical factors like substrate stiffness and geometry to the cells and creates a self-sufficient system for differentiation and spatial organization using induced pluripotent stem cells, without the need of using exogenous induction. We demonstrate that in response to substrate stiffness and colony geometry, iPS cells differentiate into and organize a SOX17+ endodermal population along with the appearance of a T/Brachyury+ population, confirmed by fluorescence imaging and qPCR results, which represents primitive streak/mesoderm. These micro-colonies control the orientation of cell packing and the confinement causes cytoskeleton mediated nuclear shape alterations, inducing the signaling dynamics to create these gastrulation-like events which are enhanced when these colonies are cultured in a 3-D environment, implicating the crucial role of mechanics of the culture substrates. This model presents a simple approach to understand how pluripotent cells interpret the intricacies of their physical microenvironment to alter cell identity and mediate spatial organization, which better represents the in-vivo environment.

Funding Source: This work was supported through funding from the Australian Research Council award # FT180100417.

Keywords: Gastrulation, Early Development hiPSCs, micropatterning Self-organization

Poster: 634

HAPLOINSUFFICIENCY DISEASE MODELLING AND ANALYSIS OF DOSAGE-DEPENDENT PATHWAYS IN HUMAN EMBRYONIC STEM CELLS

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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, its extent and molecular basis are not fully understood. To date, the effect of haploinsufficiency on human embryonic stem cell (hESC) growth and proliferation has not been thoroughly studied. Our aim is to identify genes that require two functioning alleles for normal hESC growth, and a loss-of function of one of the two alleles will affect the cells. To establish a genome-wide loss-of-function screening for heterozygous mutations, we fused a library of mutant haploid hESCs with normal haploid cells. We have identified over 600 genes with a negative effect on hESC growth in a haploinsufficiency manner and characterized them as genes that show less tolerance to mutations, more conservation over evolution and depletion from telomeres and X chromosome. Interestingly, a large portion of the essential genes with heterozygous mutation was found in extracellular matrix and plasma membrane proteins. We have discovered enrichment of haploinsufficiency disease-related genes in WNT and TGF-beta signal transduction pathways, which are needed for hESCs. We could thus identify haploinsufficiency-related disorders that show growth retardation and other molecular phenotypes even in early embryonic cells. We suggest that hESCs show dosage-dependent phenotypes in these pathways, indicating a cellular or molecular phenotype even when a single allele is mutated. Overall, we have constructed a novel model system for studying the effect of haploinsufficiency and identified important dosage-dependent pathways involved in hESC growth and survival.

Keywords: GENOME-WIDE SCREENING, HAPLOINSUFFICIENCY, DISEASE MODELLING

Poster: 635

A NOVEL IN VITRO DIFFERENTIATION PROTOCOL FOR HUMAN EMBRYONIC BIPOTENTIAL GONAD AND TESTIS CELL DEVELOPMENT

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Currently an in vitro model that fully recapitulates the human embryonic gonad is lacking. Here we describe a fully defined feeder-free protocol to generate early testis-like cells with the ability to be cultured as an organoid, from human induced pluripotent stem cells. This stepwise approach uses small molecules to mimic embryonic development, with upregulation of bipotential gonad markers (LHX9, EMX2, GATA4, and WT1) at day 10 of culture, followed by induction of testis Sertoli cell markers (SOX9, WT1, and AMH) by day 15. Aggregation into 3D structures and extended culture on Transwell filters yielded organoids with defined tissue structures and distinct Sertoli cell marker expression. These studies provide insight into human gonadal development, suggesting that a population of precursor cells may originate from a more lateral region of the mesoderm. Our protocol represents a significant advance toward generating a much needed human gonad organoid for studying disorders/differences of sex development.

Funding Source: National Health and Medical Research Council (NHMRC), Australia (APP1156942; APP1074258: APP1136085; APP1154187). The University of Melbourne, Australia. International Foundation for Ethical Research (IFER), USA.

Keywords: gonadal and testis development, Differences/ Disorders of Sex Development, organoid cultures

Poster: 636

DECREASED GLUT2 AND GLUCOSE UPTAKE CONTRIBUTE TO INSULIN SECRETION DEFECTS IN MODY3/HNF1A HIPSC-DERIVED HUMAN β CELLS

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Maturity onset diabetes of the young 3 (MODY3) are caused by heterozygous HNF1A gene mutations, and are characterized by insulin secretion defects. However, the specific mechanisms of MODY3 in humans remain unclear due to the lack of access to diseased human pancreatic cells. In this study, we utilized MODY3 patient-derived human induced pluripotent stem cells (hiPSCs) to study the effects of a causal HNF1A(+/H126D) mutation on pancreatic β cell function. Molecular dynamics simulations predicted that the H126D mutation alters the interaction of the residue with its neighbors, which could reduce the ability of HNF1A as a transcription factor to bind to DNA and activate gene transcription. Genome-wide RNA-Seq and ChIP-Seq analyses on MODY3 hiPSC-derived endocrine progenitors revealed that the HNF1A(+/H126D) mutation resulted in loss of binding and downregulation of several HNF1A gene targets that are involved in pancreas development, β cell survival and insulin secretion. We also found decreased expression of glucose transporter GLUT2, which is associated with decreased glucose uptake function and decreased ATP production in the MODY3 hiPSC-derived β-like cells. Overall, our MODY3-hiPSC-based findings reveal the importance of HNF1A in regulating several genes involved in insulin secretion function that can account for the insulin secretory defect clinically observed in MODY3

patients. Modulation of these targets could serve as a viable means to restore the insulin secretory capacity of dysfunctional MODY3 β cells.

Funding Source: A*STAR IMCB, NHG-KTPH and NMRC

Keywords: pancreatic beta cells, MODY3, HNF1A

Poster: 637

EXPANSION OF HUMAN IPSC-DERIVED URETERIC BUD ORGANOIDS WITH REPEATED BRANCHING POTENTIAL

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During mammalian kidney development, ureteric bud (UB), a progenitor tissue of renal collecting ducts, repeat branching morphogenesis. Although significant progress in kidney regeneration research using human iPS cells (hiPSCs) has been made, hiPSC-derived UB structures have limited developmental ability and show a small number of branching. Here, we improved our previous UB differentiation method and successfully generated UB organoids with epithelial polarity and tubular lumens that repeat branching morphogenesis. Furthermore, we confirmed that our UB organoids recapitulate in vivo development accompanied by spatiotemporal regulation of the gene network by high-resolution transcriptome analysis using single cell RNA sequencing, showing the reciprocal expression of UB tip and trunk markers. We also succeeded in establishing in vitro monitoring and expansion methods for UB tip cells that can efficiently reconstitute branching UB organoids and differentiate into collecting duct. Finally, we developed a disease model that reproduces some phenotypes of multicystic dysplastic kidney (MCDK) using the UB organoids. This system could be applicable to multiple hiPSC lines including patient-derived iPSCs and contribute to elucidating the developmental mechanisms of UB branching, establishing robust differentiation methods of renal collecting duct, and developing disease models for congenital renal abnormalities.

Keywords: Human iPS cell, Organoid, Ureteric bud

Poster: 638

DIFFERENTIATION OF CANINE INDUCED PLURIPOTENT STEM CELLS INTO DEFINITIVE ENDODERM

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Induced pluripotent stem cells (iPSCs) are generated from somatic cells, exhibit self- renewability, and can differentiate into all three germ layers. Therefore, iPSCs are an attractive cell resource for regenerative medicine and disease modeling, and the ability to efficiently generate definitive endoderm (DE), precursor cells of the liver, pancreas, lungs, thyroid, and intestines, is of great clinical importance. Various DE induction protocols using human iPSCs have been reported. Dogs acquire naturally occurring diseases like humans; therefore, the application of canine iPSCs (ciPSCs) is also desired. Although we have reported the generation of ciPSCs, DE induction using ciPSCs has not been reported yet. Therefore, in this study, we aimed to differentiate the in-house ciPSCs into DE. After seeding ciPSCs on Matrigel as cell clusters, we differentiated them using 100 ng/mL Activin A and increasing the gradient of FBS as follows: day 0 at 0%, day 1 at 0.2%, and day 2 at 2%, based on a previously developed protocol using human iPSCs. However, post differentiation, almost all cells died. Therefore, we compared cell viability after differentiation between the increasing gradient of FBS and FBS throughout the process (2% or 5%), and between 100 ng/mL and 10 ng/mL Activin A. The results revealed 2% FBS not Activin A is necessary for cell viability. Thereafter, we analyzed the DE marker, CXCR4, during differentiation in 100 ng/mL Activin A and 2% FBS using flow-cytometry at 0 h, 24 h, 48 h, 72 h, and 96 h and found the ratio of CXCR4-positive cells increased during the first 3 d and then reached a plateau. Previously, it was reported single cell passage of human iPSCs improved the induction efficiency of DE. Therefore, we passaged ciPSCs on Matrigel as cell clusters or single cells at 5.0 × 104, 1.0 × 105, and 1.5 × 105 cells/cm2 and differentiated in 100 ng/mL Activin A and 2% or 10% FBS for 3 d. Thus, the addition of 10% FBS improved the induction efficiency of CXCR4-positive cells. Furthermore, the combination of 10% FBS and single cell passage regardless of the starting cell number was the most effective, resulting in ratio of CD49e, which is another marker of DE, and CXCR4 double-positive cells of up to 45%. This is the first attempt of DE induction from ciPSCs, and further studies are needed for the differentiation of DE into downstream organs.

Funding Source: This work was supported by JSPS KAKENHI Grant Numbers JP19J22851, JP18K19273, JP18H02349.

Keywords: canine induced pluripotent stem cells, definitive endoderm, veterinary medicine

Poster: 639

HUMAN LIVER ORGANOID AS A 3D BILE DUCT MODEL FOR BILE DUCT CELL TOXICITY SCREENING AND BILIARY DISEASE MECHANISM STUDY

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Biliary atresia (BA) is a poorly understood and devastating obstructive bile duct disease of newborns. Plant toxin Biliatresone caused bile duct damages and BA-like syndrome in some animals but the relevance of biliatresone exposure to BA in humans is unknown. We have shown that human liver organoids favorably resemble the in-vivo bile duct development, and BA liver organoids exhibit BA-like biliary anomalies. Therefore, liver organoid is a good human proxy for bile duct toxicity evaluation and disease mechanistic study of BA. To address the relevance of biliatresone exposure on human bile duct damage and BA in humans. We treated normal human liver tissue derived organoids with biliatresone (2μg/mL), and address its adverse effects on organoids' development, morphology, cellular organization, primary cilia and cilia mediated mechano-sensory function. The control organoids (without biliatresone) were well expanded and much bigger than biliatresone treated organoids. Expression of cholangiocyte marker CK19 was drastically reduced, while hepatocyte marker HFN4A was markedly elevated in biliatresone treated organoids. ZO-1 (tight junction marker) immunoreactivity was localized at the apical intercellular

junctions in control organoids, while ZO-1 immunoreactivity was markedly reduced in biliatresone treated organoids. Cytoskeleton protein F-actin was localized at the apical surface of the control organoids, but it was ectopically expressed both at the apical and basal sides in biliatresone treated organoids. Cholangiocyte of control organoids possess primary cilia and elicited cilia mechano-sensory function. However, ciliated cholangicoyte was significantly reduced in biliatresone treated organoids and cilia mechano-sensory functions was severely hampered. Biliatresone induces morphological and developmental changes found in BA organoids, suggesting that environmental toxins could contribute to BA pathogenesis. Environmental toxin screening may be warranted for BA and other biliary diseases prevention. This novel human BA model can be used for pre-clinical testing of new drugs to improve treatment outcome of biliary diseases.

Keywords: Liver Organoids, Bile Duct Damage, Biliary Atresia

Poster: 640

LACTATE PROMOTED SURVIVAL OF INDUCED PLURIPOTETN STEM CELLS IN A MEDIUM WITHOUT GLUCOSE AND SUPPLEMENTED WITH GALACTOSE

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Glucose is essential for the survival of cells, as they tend to die in the media without glucose supplementation. Glucose is produced from galactose through gluconeogenesis, a process that occurs in the hepatocytes. Induced pluripotent stem (iPS) cells die within three days, while primary human hepatocytes can still survive in a medium without glucose and supplemented with galactose (hepatocyte selection medium, HSM). HSM is potentially useful to eliminate the residual undifferentiated iPS cells when hepatocytes are generated from them thereafter. After culturing the cells for two days in HSM, RNA was isolated from iPS cells, which was then subjected to real-time quantitative PCR analysis. Alpha-feto protein, which is a marker of immature hepatocytes, was found to be up-regulated by 10.3±1.9 folds when compared to the undifferentiated iPS cells. It is suggested that HSM unexpectedly promotes the differentiation of iPS cells to generate hepatocytes. If iPS cells survive in the glucose-deprived medium, they further differentiate to generate hepatocytes. Therefore, it was important to establish a method to help iPS cells survive under glucose-deprived condition. Metabolome analysis was performed to elucidate the metabolic differences between iPS cells cultured in HSM and those in undifferentiated state. The concentration of lactate decreased to 1/40.8 in iPS cells cultured in HSM when compared to those in undifferentiated state. iPS cells cultured in HSM supplied with calcium lactate were observed under a microscope. Precipitate formation was observed when the concentration of calcium lactate used was over 3mM. Thereafter, iPS cells cultured in HSM supplied with calcium lactate at a concentration of 1mM were observed under a microscope. The cells were viable even after seven days of culture. It indicated that calcium lactate is useful for iPS cells to survive in HSM. Therefore, our next step would be to analyze the differentiation status of iPS cells to generate hepatocyte lineage through assessing the expression of hepatocyte markers. Three-dimensional culture of iPS cells in HSM supplied with calcium lactate would also be worth elucidating.

Keywords: induced pluripotent stem cells, hepatocyte, lactate

Poster: 641

THE ROLES OF NON-CANONICAL WNT7B SIGNALING AND YY1 IN THE PROLIFERATION OF HUMAN PANCREATIC PROGENITORS REVEALED BY TRANSCRIPTOME AND PHOSPHOPROTEOME APPROACHES

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Type 1 diabetes (T1D) patients can achieve insulin independence from transplantation of cadaveric islets, but the scarcity of donor pancreatic organs limits the widespread application of this treatment. Recent efforts to differentiate human pluripotent stem cells (hESCs/iPSCs) into islet cells have led to the development of protocols that generate glucose-responsive insulin-secreting β cells for transplantation. To meet clinical demand, further manufacturing refinement is required to prepare β cells in an efficacious and cost-effective manner. Therefore, the in vitro proliferation of human pancreatic progenitor cells (PPCs), an intermediate cell type differentiated during the course of the induced differentiation of hESCs/iPSCs into β cells, is critical for developing cell therapies for diabetes. However, the mechanism of PPC growth during organogenesis or in vitro induction is incompletely understood. Here, using transcriptome analysis combined with siRNA screening, we revealed that WNT7B is a downstream growth factor of AT7867, a compound we previously identified that promotes the proliferation of PPCs derived from hESCs/iPSCs. Feeder cells stably expressing mouse/human Wnt7a or Wnt7b, but not other Wnts, enhanced proliferation of PPCs in the absence of AT7867. The pancreatic progenitors cultured in the presence of Wnt7a/b ligands maintained the expression of progenitor markers including PDX1, NKX6.1, FOXA2, SOX9 and HNF1β. Importantly, these PPCs also maintained differentiation potential into insulin-producing β cells. More analysis revealed that Wnt7a/b did not activate the canonical Wnt pathway, and our data suggest PPC proliferation depends on the non-canonical Wnt/PKC pathway. Furthermore, phosphoproteome analysis of PPCs cultured in the presence of AT7867 uncovered the role of a transcription factor Yin Yang 1 (YY1) as a regulator of WNT7B transcription. Our model suggests that AT7867 inhibits phosphorylation of YY1 at Ser118, resulting in the upregulation of WNT7B expression and the increase in PPC proliferation. Overall, our data highlight an unknown role of non-canonical WNT7B/PKC signaling in human PPC proliferation and will contribute to the stable supply of a cell source for pancreatic disease modeling and therapeutic applications for T1D.

Keywords: Pancreatic progenitor, Non-canonical Wnt/PKC signaling pathway, Yin Yang 1 (YY1)

Poster: 642

GENERATION OF QUIESCENT HEPATIC STELLATE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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By mimicking the early stage of liver organogenesis, our laboratory has developed novel organoid culture technologies to reconstruct the liver from human induced pluripotent stem cells (hiPSCs). However, owing to the lack of relevant cell types like quiescent hepatic stellate cells (HSCs), the existing liver organoids have certain limitations in liver disease modeling. Various protocols have been established to generate HSCs from hiPSCs, but those differentiated cells exhibited the characteristics of activated HSC. This study aimed at generating quiescent HSCs to develop new liver organoid and model the pathogenesis of liver diseases. Simulating the development of HSC in vivo, we proposed a protocol to differentiate hiPSCs into HSCs with high purity. Compared with passaged primary human HSCs, hiPSC-HSCs are closely like hepatic stellate cell and express significantly low αSMA and Collagen I, which suggests that hiPSC-HSCs might be at quiescent stage. Moreover, we reconstructed a functional liver organoid by co-culturing hiPSC-HSCs with hiPSC derived hepatic endoderm and endothelial progenitors, which provides promising models for drug screening of fibrosis-related liver diseases.

Funding Source: This work was supported by the JST Research Centre Network for Realization of Regenerative Medicine (No. 17bm0304002h0105), Grant-in-Aid for Research Activity Start-up (No. 20K22946)

Keywords: Hepatic stellate cells, iPSCs, liver organoid

Poster: 643

INCEPTOR IS A NOVEL REGULATOR OF INSULIN SIGNALING, SYNTHESIS AND DEGRADATION IN HUMAN IPSC-DERIVED BETA CELLS

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Impaired insulin secretion and action leads to diabetes mellitus. Furthermore, insulin receptor knockout (KO) in murine pancreatic beta cells leads to defects in first phase insulin secretion and glucose intolerance, which is phenotypically similar to what is seen in human type 2 diabetes (T2D). Thus, targeting beta cell insulin signaling, synthesis and secretion might preserve and protect beta cell function and improve diabetes treatment. Strikingly, we have recently identified a novel insulin inhibitory receptor (inceptor) as a promising target for insulin receptor sensitization in beta cells to regulate systemic glycaemia. Inceptor facilitates clathrin-mediated endocytosis of the activated insulin receptor complex and genetic and pharmacological inhibition enhances insulin receptor signaling in beta cells. Here, we aimed to translate our findings to human and to further analyze the function during human beta cell development and maturation. First, we generated a CRISPR/Cas9-mediated inceptor KO in human induced pluripotent stem cells (iPSCs) that additionally express a C-peptide-mCherry fusion protein to follow beta cell differentiation and insulin-containing secretory granule formation. Stem cell-derived beta cells (SC-beta cells) were generated from control and inceptor KO iPSC lines by a directed and step-wise differentiation. During control iPSC differentiation, inceptor expression started when pancreatic progenitors were formed and was strongly enhanced during endocrine and insulin-secreting SC-beta cell formation. Interestingly, differentiation of KO iPSCs resulted in increased SC-beta cell differentiation and survival as well as greatly increased insulin levels when compared to control iPSCs, consistent with our mouse knockout in vivo results. Mechanistically, inceptor negatively regulated insulin receptor and mTORC1 signaling, which will be further discussed at the ISSCR meeting. Taken together, we have identified a novel regulator of insulin signaling, synthesis and degradation in beta cells and pharmacological targeting may allow to protect or regenerate beta cell mass and function in diabetic patients.

Keywords: diabetes, insulin signaling, human iPSC-derived beta cells

Poster: 644

MODELING FABRY DISEASE IN KIDNEY ORGANOIDS AND ENGINEERED HEART TISSUE FROM PATIENT-DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

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Fabry disease is an inherited lysosomal storage disorder, caused by mutations in the GLA gene, encoding the enzyme alpha-Galactosidase A (aGal). Patients display a wide variety of symptoms, most predominantly the development of cardiomyopathy, kidney damage and functional decline as well as neuropathic pain and premature cerebrovascular events. Currently available animal models fail to recapitulate this complex phenotype, particularly concerning the heart and kidney involvement. The aim of this project is, hence, to exploit the recent advances in human induced pluripotent stem cells (hiPSC) and organoid differentiation in order to establish innovative human in vitro models for Fabry disease. The multicellular composition and higher degree of organization of kidney organoids enables a more complex disease modelling. We established a robust directed differentiation protocol working

for both control as well as patient derived hiPSC lines. The kidney organoids we obtained show tubular structures under bright-field microscopy and immunostaining confirmed the presence of marker proteins for different nephron segments with glomerular structures. In addition, we use already established protocols for cardiomyocyte differentiation and engineered heart tissue (EHT) formation to analyse different structural and functional aspects of the disease's phenotype. So far, we collected primary urinary cells from 15 Fabry patients. Out of these, 6 were reprogrammed into hiPSC. All samples depict decreased aGal enzyme activity. Production of isogenic controls, i.e. correction of the Fabry-specific mutation in the aGal gene by CRISPR/Cas9-mediated genome editing, is under progress. Further analyses will assess the accumulation of globotriaosylceramide, the prime substrate to aGal as well as include in-depth structural and single cell analyses of Fabry kidney organoids and functional phenotype of Fabry EHTs.

Funding Source: This study was supported by the 3R (Replace, Reduce, Refine) Start-up Funding Program, awarded by the Medical Faculty Hamburg to AH and FB, and by Amicus Therapeutics through an investigator-initiated proposal awarded to FB and TBH.

Keywords: Fabry disease, kidney organoids, engineered heart tissue

Poster: 645

DECIPHERING CELL-CELL COMMUNICATION IN EARLY BLOOD VESSEL FORMATION IN IN VITRO CO-CULTURE SYSTEM VIA SINGLE-CELL SEQUENCING

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Blood vessel formation requires highly regulated cell-cell communication between endothelial cells (ECs) and mural cells such as smooth muscle cells (SMCs) and fibroblasts (FIBs). Dysregulation in blood vessel formation leads to the onset or progression of numerous diseases such as ischemic disease, cancer, inflammatory and immune disorders. Furthermore, in the field of regenerative medicine, there is a need for engineered blood vessels and the formation of blood vessels in organoids. Hence, we have developed an in vitro co-culture system to study the underlying cell-cell communication in early blood vessel formation. In this study, we used a model of co-culture system containing three cell types ECs, SMCs and FIBs mimicking the in vivo cell type composition of blood vessels compared to the commonly used system consisting of two cell types either EC and SMC or EC and Fib only. Next, we used single-cell hashing and cell tagging followed by single-cell sequencing to track ECs, SMCs and FIBs in the co-culture system. We identified key cell-cell communication patterns between ECs, SMCs and FIBs, and also master transcription factors that regulate the formation of early blood vessels. Additionally, we studied the effects of TGFB stimulation on the co-culture system and its impact on cell-cell communication in early blood vessel formation.

Keywords: Blood vessel formation, endothelial cells, co-culture system

Poster: 646

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL BIOMARKER ASSOCIATED WITH STEM CELLS IN LUMINAL BREAST CANCER.

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Amongst breast cancer (BC) subtypes, Luminal BC which is identified by distinct overexpression of hormonal receptors, estrogen (ER) and/or progesterone (PR) accounts for more than 70% of all breast cancer cases. Despite surgical intervention and adjuvant hormone therapies targeting ER and PR along with cyclin dependent kinases (CDK4/6) inhibitors, approximately 40% of tumors eventually progress further leading to possibly mortality, making it the leading cause of BC deaths. In BC it is hypothesis that a small number of invasive and motile tumor cells with stem cell like properties (breast cancer stem cells or BCSCs) are responsible for tumor initiation, immune surveillance escape, treatment resistance and eventual metastasis. Identification of BCSCs especially in luminal BC remains a major challenge due to a lack of biomarkers which can identify BCSC niches or subpopulations. Our analysis of publicly available bulk and single cell RNA-Sequencing (scRNA-Seq) show that an under-researched long non coding RNA (lncRNA)RP11-428L9.2 located on Chromosome 10 at bases 9,012,088-9,015,431 in the GATA3 gene locus is overexpressed specifically in luminal BC cells with stem cell like gene expression signature. In Luminal BC, disease free survival of patients with high expression of lncRNA RP11-428L9.2 (RP11-428L9.2High) was significantly lower than the group with low RP11-428L9.2 (RP11-428L9.2low) expression. Expression profile in tumor samples (n =21), cell lines (n=5), explants of matching tumor and surrounding tissue (n=9) and normal breast (n=3) showed high expression correlation with markers of pluripotency, invasiveness and migration. Our results of in-vitro knockout and overexpression experiments show that RP11-428L9.2High cells positively correlate with higher migration and invasion rates. Functionally, Chromatin immunoprecipitation experiments showed RP11-428L9.2's involvement at GATA3 transcription site. GATA3 is a transcription factor with a wide biological role and possible involvement in breast cancer. Our work suggests that lncRNA RP11-428L9.2 is overexpressed in luminal BCSCs with a possible role in regulating the expression of GATA3.

Keywords: luminal Breast cancer, lncRNA, Biomarker

Poster: 647

DIFFERENTIATION AND CHARACTERIZATION OF AML-M5-SPECIFIC IPSC INTO MONOCYTIC CELLS USING THE MODIFIED IN VITRO EB-BASED HEMATOPOIETIC DIFFERENTIATION PROTOCOL

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Pathogenesis of acute monocytic leukemia (AML-M5) remains largely unknown and disease-specific induced pluripotent stem cells (iPSC) can be a useful platform for disease modeling. Differentiation of disease-specific iPSC to the respective mature forms may allow better understanding of the stepwise

progression of a particular disease. An iPSC clone was previously generated by our group from AML-M5-specific cells, THP-1 cells, harboring the MLL rearrangements. In this study, we aim to differentiate the AML-M5-specific iPSC into monocytic lineage cells of various maturation stages using the modified version of In vitro Embryoid Bodies (EBs)-based protocol supplemented with interleukin-3 (IL-3) and macrophage colony stimulating factors (M-CSF). The EBs derived from AML-M5-specific iPSC were first treated with both the IL-3 and M-CSF to form circular, small non adherent monocytic lineage cells known as Myeloid Cells Forming Complexes (MCFCs). Next, MCSF was used solely to terminally differentiate the MCFCs into the monocytic lineage cells, which were then characterized using 6-colour multicolor immunophenotyping. The expression of the early hematopoietic stem cell markers includes CD34, CD38, CD133 were predominantly found in the pluripotent iPSC, Embryonic Stem Cells (ESC-H9) and early differentiated EB. However, the CD34 expression was found to be gradually lost in the differentiation culture which could be because of the presence of IL-3 as reported in other studies. Using the modified protocol, ESC-H9 (Control) were successfully differentiated into the late monocytic cells, monoblast, promonocyte and monocytes, which showed co-expression of various hematopoietic/monocytic markers including HLA-DR, CD4, CD117, CD14 and CD68. Same protocol was used to differentiate the AML-M5 specific iPSC but a relatively smaller population of promonocytes and monocytes which co-expressing HLA-DR, CD4, CD117 and CD68 were detected as compared to ESC. Further characterization assays are needed to conclude whether the AML-M5-specific iPSC regain the leukemic potential causing monocytic cell differentiation arrest.

Funding Source: The research project is funded by Fundamental Research Grant Scheme (FRGS) and National Cancer Council (MAKNA).

Keywords: Induced pluripotent stem cell, Acute monocytic/monoblastic leukemia, Hematopoietic/Monocytic differentiation

Poster: 648

BIDIRECTIONAL NUCLEAR TRANSPORTER IMPORTIN 13 IS CRITICAL FOR CELL SURVIVAL DURING EMBRYONIC STEM CELL DIFFERENTIATION

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Nuclear transporter Importin (Imp, Ipo) 13 is known to transport various mammalian cargoes into/out of the nucleus, but its role in directing cell-fate is unclear. Here we examine the role of Imp13 in the maintenance of pluripotency and differentiation of embryonic stem cells (ESCs) for the first time, using an embryonic body (EB)-based model. When induced to differentiate, Ipo13-/- ESCs displayed slow proliferation, reduced EB size, and lower expression of the proliferation marker KI67, concomitant with an increase in the number of TUNEL+ nuclei compared to wildtype ESCs. At days 5 and 10 of differentiation, Ipo13-/- EBs also showed enhanced loss of the pluripotency transcript OCT3/4, and barely detectable clusters of OCT3/4 positive cells. Day 5 Ipo13-/- EBs further exhibited reduced levels of the mesodermal markers Brachyury and Mixl1, correlating with reduced numbers of haemoglobinised cells generated. Our findings suggest that Imp13 is critical to ESC survival as well as early post-gastrulation differentiation.

Keywords: Embryonic Stem Cells, Importin 13, Preliminary blood cells

Poster: 649

3D GENOME REORGANIZATION IN MOUSE HEMATOPOIETIC STEM CELLS DURING AGING

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Declined function of hematopoietic stem cells (HSCs) markedly contributes to hematopoietic diseases in the elderly. Recent studies have uncovered 3D genome organization regulates essential cellular process such as stem cell differentiation and cell senesce. However, higher-order change during aging remains elusive. To explore the molecular feature associated with the functional decline of HSC, we sorted HSCs and hematopoietic progenitor cells (HPSCs) from young and old mice by flow cytometry. We used low-input Hi-C approach to exam the chromatin architecture recognition during aging. In combined with the transcriptome and histone modification profiles, we provided the multiple layers of regulation in stem cell aging.

Keywords: HSCs, 3D genome organization, Aging

Poster: 650

RECAPITULATION OF HEPATIC HEMATOPOIESIS WITH HUMAN LIVER ORGANOIDS

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The fetal liver is a major hematopoietic organ during embryo development, and hematopoietic progenitors generated from the yolk sac and aorta-gonad-mesonephros region contribute to embryonic hematopoiesis in the fetal liver. However, the interactions between human liver organogenesis and hepatic hematopoiesis are still unclear. This study aims to recapitulate the early hepatic hematopoiesis with human induced pluripotent stem cells (hiPSCs) derived liver organoids. First, we developed a feeder-free method to generate hematopoietic progenitors from hiPSCs. These hematopoietic progenitors were defined by the expression of specific surface markers and resembled erythro-myeloid progenitors (EMPs) originating from the yolk sac. Moreover, these hiPSC-derived EMP-like cells hold the potential to differentiate into multiple downstream hematopoietic cells, including monocyte, granulocyte, megakaryocyte, and erythrocyte. To mimic the early hematopoiesis during liver development, we established liver organoids composed of

hiPSC-derived EMP-like cells, hepatic endoderm, endothelial progenitors, and mesenchymal progenitors. During the maturation of liver organoids, we noticed a significant proliferation of hematopoietic cells, and these proliferated hematopoietic cells differentiated into the myeloid lineages and megakaryocyte-erythrocyte lineages. Notably, the cellular proliferation and differentiation were not detected in EMP-like cells culture alone. Taken together, we ex vivo recapitulated EMP-mediated embryonic hematopoiesis in liver organoids, which would help to explore the underlying mechanisms between liver organogenesis and hepatic hematopoiesis in future.

Keywords: Hepatic hematopoiesis, Erythro-myeloid progenitors (EMPs), Liver organoids

Poster: 651

SCALABLE PRODUCTION OF IPSC-DERIVED MICROGLIA FOR MODELING NEUROINFLAMMATION

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Identification of genetic risk variants impacting neuroinflammation in neurodegenerative diseases has caused a growing interest in microglial studies. This, in turn, has generated a need for scalable production of iPSC-derived microglia-like cells that reflect typical pathophysiological properties of primary cells. During development, microglial precursors arise from embryonic hematopoiesis and migrate into the developing central nervous system. We mimicked this developmental sequence by devising an in vitro protocol enabling parallel generation of yolk sac derivatives and neuroepithelial cells within differentiating aggregates. Starting from week 4, this multilineage differentiation culture releases microglial cells (iPSdMiG) into the supernatant, from which they can be easily and repeatedly harvested. For scalable production, we combined the 3D iPSC aggregation step in a table-top bioreactor with a subsequent carrier-based suspension culture that facilitates retrieval of the produced microglia at high purities of >90 %. Cells could be harvested weekly over 7 weeks with 1 x 106 iPSCs typically giving rise to 15 x 106 iPSdMiG. These displayed robust microglia-specific marker expression, secretome profiles, inducible phagocytosis and radical production across genetically distinct production batches. These biological properties were maintained following cryoconservation. In silico comparison to primary adult human microglia confirmed a high similarity, with 18 of the top 20 enriched core transcription factors overlapping. Furthermore, we analyzed the suitability of these iPSdMiG for studying NLRP3 activation, a pharmaceutically interesting target associated with numerous inflammatory diseases. ASC-specks generated via NLRP3 inflammasome activation in microglia have been shown to enhance amyloid beta aggregation in mouse models, leading to disease exacerbation. Here we show that in iPSdMiG, NLRP3 inflammasome activation occurs via a canonical 2-step process that can be mimicked by LPS-driven priming and subsequent nigericin activation, leading to ASC speck formation. We expect this protocol to facilitate large-scale production of iPSdMiG for disease modeling and pharmaceutical studies into mitigating neuroinflammation-associated damage in neurodegenerative disease.

Funding Source: Innovative Medicines Initiative 2 Joint Undertaking, Grant/Award Number: PHAGO no. 115976

Keywords: iPS cell-derived microglia, multilineage differentiation, NLRP3 inflammasome

Poster: 652

DETECTION OF ANTIBODY-DEPENDENT ENHANCEMENT (ADE) ACTIVITY IN SERUM DERIVED FROM SARS-COV-2 INFECTED PATIENTS USING HUMAN IPS-DERIVED AND IMMORTALIZED MYELOID CELL LINES

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Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many vaccine trials are ongoing. One of the important goals of the vaccine is the development of neutralizing antibody (Ab) against SARS-CoV-2. However, possible induction of antibody-dependent enhancement (ADE) of infection, which is known for other coronaviruses and dengue virus infections, is a particular concern in vaccine development. So far, there is no reports on the in vitro systems to evaluate Abs for ADE-causing potentials upon the infection of live SARS-CoV-2. Here, we demonstrate that human iPS cell-derived and immortalized myeloid cell lines, named Mylc lines, are useful as host cells in SARS-CoV-2 infection. Furthermore, using Mylc lines, it is possible to evaluate the potentials of serums from severe COVID-19 patients to cause ADE and to stimulate IL-6 production upon the infection with SARS-CoV-2. These data suggest that Mylc lines would serve in monitoring the ability of Abs (neutralizing or ADE-causing) induced by vaccine candidates.

Keywords: SARS-CoV-2, antibody-dependent enhancement, IL-6

Poster: 653

GENERATION AND CHARACTERISATION OF POST-REPROGRAMMED HUMAN OSTEOSARCOMA, POST-IG-292

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Osteosarcoma is a common primary bone tumour diagnosed in children and adolescents, affecting the patients during their productive years. Current treatments for osteosarcoma are associated with significant morbidity and substantial loss of quality of life for patients in the prime of their life. The aetiology for osteosarcoma remains unknown and is it essential to understand the genetic changes during the differentiation process, that could provide valuable insight into the pathogenesis of osteosarcoma, especially in recurrent and metastatic cases. The advancement in the field of induced pluripotent stem cells (iPSC) provided a mean to study osteosarcoma pathogenesis. Reprogramming of

osteosarcoma cells to a more primitive stage could be used for disease modelling. We have successfully generated an iPSC line, known as iG-292, from an osteosarcoma cell line, G-292, using the Yamanaka factors. The generated iG-292 was re-differentiated back to the parental stage by culturing in the same medium used for G-292 maintenance. Morphological observations showed a difference between the post-iG-292 cells from the parental G-292. The post-iG-292 demonstrated a slower proliferation when compared with parental G-292. We postulated that the post-iG-292 cells are more primitive than G-292. However, further investigation on the characteristics of post-iG-292, especially the migration and invasion assays, and molecular profiling are needed to understand these population. This study aims to gather valuable and novel information on osteosarcoma pathogenesis using a disease modelling approach.

Funding Source: The research was funded by Nasional Cancer Council Malaysia and the work was done at Universiti Tunku Abdul Rahman.

Keywords: cancer reprogramming, osteosarcoma, disease modelling

Poster: 654

DETECTION OF COMPOUNDS THAT AMELIORATE DECLINES IN CONTRACTILE PERFORMANCES IN DMD BY THE OVERUSED MUSCLE TRAINING MODEL USING PATIENT-DERIVED IPSCS IN VITRO BY THE SMALL-SCALE SCREENING

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Duchenne muscular dystrophy (DMD) is a progressive muscle degenerating disease caused by a loss of dystrophin protein, and therapeutics are quite limited. DMD is characterized by the decline of muscle performance accompanied with muscle fatigue leading to the overused muscles, eventually causing the degeneration of muscle fibers as an onset of the disease. Those muscle specific-primary phenotypes are followed by the secondary phenotypes such as inflammatory responses. A current limitation of drug discovery in DMD is a lack of appropriate in vitro model that recapitulates a decline of muscle performance and muscle fatigue. An establishment of such as a model will accelerate identifying a target molecule to prevent the initiation of DMD and developing a strategy of drug development. Previously, we have established a combinational myogenic culture system using hiPSCs with electrical-field stimulation (EFS) and collagen gel. Myotubes cultured in this system showed a progressed myogenic maturation characterized by sarcomere formation and a response of excitation-contraction coupling. Using the system, we recapitulated a muscle fatigue-like decline in contractile performances without cellular damages by the EFS training program (1Hz, 20V, 2ms, continuous for 2 weeks) using DMD-Δ44 and DMDΔ46-47 iPSCs, representing an overused muscle. In this study, we conducted a small-scale screening using clinical-related compounds such as a deflazacort and commercially available Ca2+ modulators. From the screening, we detected three compounds that ameliorate the decline in contractile performances. Since all of them modulates Ca2+ influx by interacting different gene of targets, Ca2+ mobilization along excitation-contraction coupling may be a key to regulate contractile performances. On the other hand, most of clinical compounds failed to rescue the phenotype. We consider it because of different mechanisms of actions or absence of target cells of compounds such as immune cells in our model. Nevertheless, our data indicated that skeletal muscle itself is sufficient to develop a contractile phenotype, and targeting it may have a clinical potential to develop a new drug that has a different mechanism of actions compared to existing drugs.

Funding Source: This work was funded by Takeda Pharmaceutical Company Limited.

Keywords: Duchenne Muscular Dystrophy, iPSCs, Muscle fatigue

Poster: 655

MODELING LIMB SKELETOGENESIS IN ORGANOID CULTURE DERIVED FROM MOUSE EMBRYONIC LIMB BUD AND HUMAN ES CELLS

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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. Interestingly, after manipulation of positional identities of those cells by cytokines, those aggregates showed different patterns of cartilage formation and its growth. Those differences might reflect position-specific cellular properties which contribute to position-specific morphogenesis during limb development. 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 and derived cells which express typical limb mesenchyme marker genes. Those cells were able to form cartilage in 3-dimensional culture. We expect our study would establish novel model system to assess causality of human congenital limb deficiencies. Furthermore, we recently reported the derivation of limb bud cells from mouse embryonic stem cells. Establishment of self-organizing system of limb skeletal morphogenesis from pluripotent stem cells of multiple species would open up venues to understand cellular mechanisms of species-specific limb morphologies.

Keywords: Limb skeletal morphogenesis, Organoid, Human embryonic stem cells

Poster: 656

EXPLORING HUMAN SKELETAL DEVELOPMENT USING A HUMAN BONE TISSUE INDUCED FROM HUMAN PLURIPOTENT STEM CELLS

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Further elucidation of the complex and spatiotemporal mechanism of human skeletal development is crucial for understanding the skeletal system, and could lead to improvements in clinical interventions. Human pluripotent stem cells (hPSCs) are an attractive tool to investigate human tissues and development. We sought to develop a method to recapitulate the human bone formation process using hPSCs. We optimized a stepwise protocol to induce the sclerotome from hPSCs in vitro and then implanted the induced sclerotome into renal capsules of immunodeficient mice, in order to induce three-dimensional bone formation. In vivo micro-CT scanning showed continuous growth of calcified tissues. In histological analysis, the tissues showed endochondral bone-like structures composed of cartilage with a well-organized alignment of columnar and hypertrophic chondrocytes, bone collars, and bone marrows. Immunostaining confirmed specific expressions of osteogenic and chondrogenic markers in each component. To examine the developmental dynamics of the endochondral bone-like tissues, we applied time-course scRNA-seq to the total of 11,354 cells isolated from the tissues. Clustering analysis identified species-specific cell clusters with distinct signatures of skeletal components; clusters representing chondrocytes and osteoblasts were derived from human cells, whereas those representing blood vessels, monocytes, and osteoclasts were from mouse cells. Pseudotime analysis predicted a pathway for osteoblast and chondrocyte differentiation from human osteo-chondroprogenitors. Ligand-receptor analysis revealed that key signaling pathways in endochondral ossification, such as those involving BMP and Hedgehog, were conserved in the induced tissues. Gene regulatory analysis further identified novel transcriptional regulators expressed in distinct patterns during skeletal development. The contribution of these factors to human bone development was confirmed in vitro. Collectively, the results showed that our induction method recapitulates the human endochondral ossification process and provides a platform to investigate human skeletal development. This is an important first step in unraveling the regulatory mechanism in human skeletal development with an hPSC-based approach.

Keywords: human pluripotent stem cells, human skeletal development, single cell transcriptome

Poster: 658

MICRORNA CONTROL IN THE REDUCED OSTEOGENESIS BUT INCREASED ADIPOGENESIS IN THE BONE MARROW FOLLOWING METHOTREXATE TREATMENT IN RATS

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Methotrexate (MTX) is commonly used in cancer chemotherapy to treat childhood leukaemia and osteosarcoma. Although the application of MTX chemotherapy improves the population of survivors, the prevalence of chronic bone-related complications has increased. Reduced bone formation (osteogenesis) and increased marrow fat formation (adipogenesis) have been observed through a “switch-like” change in commitment of bone marrow stromal cells (BMSCs) following MTX treatment. However, the underlying molecular mechanisms of this bone/fat switch are not fully elucidated. MicroRNAs participate in regulating BMSC differentiation by targeting the 3’ untranslated region of osteogenesis/adipogenesis related genes. Here, in bone samples from MTX-treated rats, we found some specific differentially expressed microRNAs by microRNA array and RT-PCR. Target prediction and dual-luciferase assays indicated that Smad2 and sFRP-1 as the direct targets for microRNA-6315 and microRNA-542-3p, respectively. In vitro cell models were applied to validate the effects of microRNA agomir and antagomir delivery. Results suggest that these microRNAs are positively correlated to osteogenic differentiation but negatively associated with adipogenic differentiation. Subsequent signalling studies have shown that microRNA-6315 can regulate osteogenesis and adipogenesis through TGF-β/Smad2 signalling, while microRNA-542-3p modulates bone and marrow fat formation through Wnt/β-catenin signalling. Collectively, our findings suggest that these microRNAs have a regulatory role in MTX treatment-induced bone marrow bone/fat imbalance.

Keywords: Methotrexate, BMSC differentiation, microRNAs

Poster: 659

EXAMINATION OF ALPHA-SYNUCLEIN DYNAMICS IN INDUCED PLURIPOTENT STEM CELL-DERIVED DOPAMINERGIC NEURONS FROM A PARKINSON’S DISEASE PATIENT (PARK4) WITH SNCA TRIPLICATION

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Parkinson’s disease (PD) is a neurodegenerative disorder caused by the selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). Although studies

on familial forms of PD have elucidated the chromosomal loci of causative genes (PARK1-23), sporadic forms of PD with unknown mechanisms contribute to approximately 90% of the PD cases. Lewy bodies (LBs), which are primarily composed of α-synuclein protein, are formed in the SNc of most patients with either familial or sporadic PD, except for certain patients with familial PD carrying PARK2 (PRKN; parkin RBR E3 ubiquitin protein ligase) and PARK8 (LRRK2; leucin-rich repeat kinase 2). Although the mechanism underlying α-synuclein aggregation in LBs is unknown, therapies aimed at reducing α-synuclein levels have been investigated, including the use of antisense oligonucleotides (ASOs) and α-synuclein-specific antibodies. In PARK4 patients, α-synuclein gene (SNCA) multiplication is observed without any pathological mutation, even though their symptoms develop relatively early. Therefore, PARK4 might be useful in investigating the mechanism through which DA neurons regulate α-synuclein aggregation before the clinical onset of PD, and could lead to the development of new therapies for patients with sporadic or familial PD. In this study, to examine the effects of α-synuclein aggregation on DA neurons, we investigated the dynamics of α-synuclein in DA neurons differentiated from human induced pluripotent stem cells (hiPSCs) derived from a PARK4 patient with SNCA triplication and a healthy donor. Furthermore, we examined the feasibility of a therapeutic strategy to reduce α-synuclein levels using ASOs.

Keywords: Parkinson's disease, SNCA triplication, human induced pluripotent stem cell

Poster: 660

TROPHOBLAST GLYCOPROTEIN: A NOVEL GENETIC FACTOR ASSOCIATED WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is a movement disorder caused by the progressive degeneration of the midbrain dopaminergic (mDA) neurons in the substantia nigra pars compacta (SNc). Despite intense research efforts over the past decades, the etiology of PD remains largely unknown. Here, we discovered the involvement of trophoblast glycoprotein (Tpbg) in the development of PD. Expression of Tpbg was detected in the ventral midbrain during embryonic development and was maintained in mDA neurons of the SNc and ventral tegmental area in adulthood. Genetic ablation of Tpbg resulted in a mild degeneration of mDA neurons in aged mice (12–14 months) with increased number of activated microglia, enhanced accumulation of alpha-synuclein and evident apoptotic cell death. In their old age, Tpbg-mutant mice also showed a decline in striatal dopamine contents and behavioral impairments reminiscent of PD-like symptoms. In silico network analysis of the interaction partners of TPBG identified several proteins whose functions were relevant to PD pathogenesis. These findings indicate that dysfunction of Tpbg contributes to PD pathogenesis as a genetic risk factor and suggest that TPBG may serve as a new molecular target for the therapeutics of PD.

Funding Source: This work was supported by Korea Health Technology R&D Project Grant (HI18C0829) through the Korea Health Industry Development Institute, founded by the Ministry of Health & Welfare

Keywords: Parkinson's disease, trophoblast glycoprotein, midbrain dopaminergic neurons

Poster: 661

A ROLE OF NEURAL STEM CELLS IN ELECTRICAL ACTIVITY OF HIPPOCAMPAL NEURONAL NETWORK

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Adult neurogenesis, where neural stem cells (NSCs) give rise to neurons and glia continuously throughout life, occurs in the hippocampus. Although previous researches have shown the relationship between the number of NSCs and learning and memory functions in the hippocampus, the mechanisms underlying how NSCs work in a pre-existing neuronal network are still elusive. Here, we investigated the role of NSCs in the electrical activity of the hippocampal neuronal network. Using high-density microelectrode arrays, which have 120 × 220 electrodes within 2 × 4 mm^2, we recorded and analyzed the spontaneous activity of the neuronal network. To prevent NSCs from proliferating and drive differentiation of NSCs in the hippocampal neuronal network, we inhibited Notch signaling by administrating DAPT to the network at an early cultural time. Changes in neuronal activities over the time course were compared between the network with NSCs (the control group) and without NSCs (the DAPT group). The synchronicity in spikes was evaluated by calculating cross-correlations between pairs of electrodes at different distances. We found that the DAPT group showed a lower degree of synchronization than the control group among distant electrodes over culture time. Furthermore, we analyzed the synchronous spikes spatially distributed across multiple recording channels called a network burst. It is known that network bursts consist of a limited number of patterns, and thus we clustered network bursts by focusing on the center of activity trajectory. We found that the DAPT group showed the same patterns over culture time, comparing with the control groups that showed different patterns. These results suggest that NSCs might involve making far functional connections among neurons in the network. Thus, the network without NSCs showed the same neuronal activity patterns caused by a relatively limited close connection between neurons. Overall, these findings show a possibility that NSCs would contribute to the more diverse neuronal activity in the hippocampal network.

Funding Source: The work was supported by the Japan Society for the Promotion of Science through Grants-in-Aid for Scientific Research (19H04437 and 19H05323).

Keywords: Neural stem cells (NSCs), High-density microelectrode array (HD-MEA), Neuronal network activity

Poster: 663

A NOVEL GENE-INTERGENIC FUSION IDENTIFIED IN DHMN1 IPSC-DERIVED MOTOR NEURONS: A NOVEL DISEASE MECHANISM IN MOTOR NEURON DISEASES?

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The distal hereditary motor neuropathies (dHMN) are a group of inherited neurodegenerative diseases with length-dependent axonal degeneration of the lower motor neurons leading to chronic disability. Our group has previously reported the pathogenic 1.35 Mb complex insertion mutation causing an autosomal dominant form of dHMN (DHMN1: OMIM %182960) in a large Australian family (Family-54). Here, we report a novel intergenic-gene fusion arising from the 1.35 Mb insertion. This finding represents a novel disease mechanism in motor neuron diseases. We have generated induced pluripotent stem cell derived motor neurons (iPSC-MN) from DHMN1 patients (n= 3) and controls (n=3) and have utilised RNA-sequencing to shed light on the patient transcriptome and potential functional pathways leading to axonal degeneration in DHMN1. Transcriptome data supported previous findings from our lab showing gene dysregulation caused by the 1.35 Mb insertion and has helped to further refine the list of potential causative candidate genes (MNX1, LMBR1, SHH, TMEM176B, LINC01006, UBE3C). Furthermore, we have identified a novel intergenic-gene fusion ('UBE3C-IntFus') involving the partial transcript of UBE3C (located in the DHMN1 insertion) fusing with upstream intergenic sequence within the DHMN1 locus. The presence of an in-frame canonical splice donor site within this intergenic sequence incorporated a pseudo-exon from the intergenic DNA sequence resulting in a novel aberrant fusion transcript being present in all the patients and absent from controls. This novel fusion transcript was validated in all patient iPSC-MN using Sanger sequencing of reverse transcribed template. Although gene dysregulation cannot yet be excluded as a contributing factor of DHMN1 axonal degeneration, identification of this novel fusion transcript represents the primary pathogenic candidate for DHMN1. Follow-up studies using both in vivo (C. elegans) and in vitro systems will be essential for understanding the contribution of UBE3C-IntFus to DHMN1 pathogenesis. *Authors contributed equally to this work.

Keywords: iPSC, motor neurons, genetics

Poster: 664

EXPLORING GENE DYSREGULATION IN CMTX3 NEUROPATHY USING PATIENT-DERIVED PERIPHERAL NERVE TISSUE

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Charcot-Marie-Tooth (CMT) neuropathy is a genetic condition that causes the degeneration of peripheral nerve fibres. With mutations in over 100 genes known to cause CMT, it is difficult to identify treatments for this heterogenous disease. CMTX3 is a rare, X-linked form of CMT neuropathy. Previously, our group identified a structural variation mutation as the cause of CMTX3. The mutation was characterised as an interchromosomal insertion whereby 78 kb of chromosome 8q24.3 has been inserted into a gene desert at chromosome Xq27.1. However, the pathogenic consequence of this mutation remains unsolved. We hypothesise that the CMTX3 insertion may cause neuropathy via altering spatiotemporal regulation of nearby genes. In line with this, our group has previously shown that CMTX3 patient lymphoblasts show dysregulated expression of FGF13; a gene located ~1.2 Mb away from the pathogenic insertion. However, given the tissue-specific nature of transcriptional regulation, it is crucial that gene dysregulation is assessed within disease-relevant tissue. Thus, we have utilised induced pluripotent stem cells (iPSC) derived from CMTX3 patient fibroblasts to generate spinal motor neurons. We first verified the pluripotency of the iPSC by assessing expression of the common pluripotency markers NANOG, OCT4 and SOX2. Subsequent differentiation of iPSC into mature motor neurons was confirmed by the expression of HB9. We then performed an exploratory RNA-sequencing experiment, comparing 1 patient sample to 3 controls, to look for genes showing differential expression in neuronal tissue. Interestingly, the preliminary results show similar levels of FGF13 expression between patients and control samples, in contrast to the FGF13 dysregulation previously observed in patient lymphoblasts. Potential candidate genes identified in this analysis will undergo further investigation to identify the specific gene dysregulation causing CMTX3 neuropathy. CMTX3 is an excellent disease paradigm to enhance our understanding of the contribution of structural variation to transcriptional dysregulation, which is an important yet critically understudied mechanism of genetic disease.

Keywords: Charcot-Marie-Tooth neuropathy, Gene regulation, Structural variation

Poster: 665

USING BINARY COLLOIDAL CRYSTALS (BCCS) AND CRISPR ACTIVATION TO IMPROVE NEURONAL DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Neurodegeneration, caused by disease or trauma, results in permanent damages to the nervous system and the deterioration of a broad set of body functions. Human induced pluripotent stem cells (iPSCs) represent a promising avenue for the development of neural regeneration strategies, by providing an unlimited source of neurons in vitro. However, conventional methods of neuronal differentiation for human iPSCs are tedious and complicated, involving multi-stage protocols with complex cocktails of growth factors and small molecules. To address this, biophysical cues from artificial extracellular matrices with defined surface topography and chemistry, could be explored as an alternative approach to improve neuronal differentiation in vitro. In the present study, we tested the effect of a type of colloidal self-assembled patterns called binary colloidal crystals (BCCs) in neuronal differentiation. To induce a rapid differentiation, we developed a CRISPR activation (CRISPRa) iPSC platform that constitutively expresses the dCas9-VPR system, which allows the robust activation of proneural transcription factors and subsequent generation of neurons within seven days. We show that the combinatorial use of BCCs can further improve this neuronal differentiation system. Our results indicate that fine tuning of silica and polystyrene size is critical to generate specific topographies that can enhance neuronal differentiation and branching. BCCs with 5 µm silica and 100 nm carboxylated polystyrene have the most prominent effect on increasing neurite outgrowth and more complex ramification, while BCCs with 2 µm silica and 65nm carboxylated polystyrene are better in promoting neuronal fate enrichment. These results indicate that biophysical cues can support rapid differentiation and improve neuronal maturation. In summary, our combinatorial approach of CRISPRa and BCCs provides a robust and rapid pipeline for in vitro production of human neurons. Furthermore, specific BCCs can be potentially adapted to late stages of neuronal differentiation protocols to improve neuronal maturation, which has important implications in tissue engineering, in vitro biological studies and disease modelling.

Funding Source: Melbourne Research Scholarship

Keywords: Neuronal differentiation, CRISPR activation, Artificial extracellular matrix

Poster: 666

EXPRESSION OF ACE2 AND A VIRAL VIRULENCE-REGULATING FACTOR CCN FAMILY MEMBER 1 IN HUMAN IPSC-DERIVED NEURAL CELLS: IMPLICATIONS FOR COVID-19-RELATED CNS DISORDERS

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It has been reported that coronavirus disease 2019 (COVID-19) causes not only pneumonia but also systemic inflammations including central nervous system (CNS) disorders. However, little is known about the mechanism that triggers the COVID-19 associated CNS disorders. In this study, we first analyzed the database and found that the expression level of SARS-CoV-2 receptor, angiotensin converting enzyme-2 (ACE2), and a viral virulent factor CCN family member 1 (CCN1) were high in the lesions of encephalopathy caused by COVID-19. A case of meningitis due to COVID-19, in which SARS-CoV-2 had been detected only in cerebrospinal fluid, has been reported, and our database analysis revealed that the expression level of ACE2 and CCN1 were also high in the choroid plexus that produces cerebrospinal fluid. Furthermore, reanalysis of existing COVID-19 studies revealed that CCN1 expression was elevated in cells and tissues other than the brain after SARS-CoV-2 infection. Considering the role of CCN1 which is known to be involved in viral toxicity and inflammation, human iPSCs-derived neural stem/progenitor cells (hiPSCs-NS/PCs) could provide an excellent model for COVID-19 associated CNS disorders from the aspect of SARS-CoV-2 infection-ACE2-CCN1 axis. And by our experiments, the expression of ACE2 and CCN1 could be confirmed in hiPSCs-NS/PCs and neurons derived from human iPSCs. From the above, it was considered that ACE2 and CCN1 may be involved in the pathogenicity of COVID-19-related CNS disorders. Finally, RNA-seq was performed on hiPSCs-NS/PCs, and it was elucidated that compound 34 and DAPT, which are γ-secretase inhibitors, have an effect of suppressing the expression of CCN1, which is considered to be a virulence factor. Collectively, our study using hiPSCs-NS/PCs may aid in the development of a therapeutic target for COVID-19-related CNS disorders.

Keywords: coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), COVID-19-related CNS disorder, human induced pluripotent stem cells (hiPSCs), human iPSCs-derived neural stem/progenitor cells (hiPSCs-NS/PCs), γ-secretase inhibitor (GSI)

Poster: 667

HIGHLY EFFICIENT DIFFERENTIATION METHODS FOR THE CENTRAL NERVOUS SYSTEM USING HUMAN TET-1-IPS CELLS

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Human pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are a promising tool for regenerative medicine, drug discovery,

developmental study, and disease modeling. To utilize them in various fields, we need to understand human embryology and properly differentiate them to a target cell type. We have investigated characteristics of uniquely-developed iPSCs with high differentiation ability (T-iPSCs) produced with Yamanaka factors and TET-1, which is involved in DNA demethylation. T-iPSCs have demonstrated high differentiation ability in light of "default differentiation", which is a bona fide program evolutionally preserved in vertebrate embryonic cells. In this study, using T-iPSCs, we produced new differentiation methods to target various brain regions with high fidelity. After carefully reviewing previous neuronal differentiation protocols, we succeeded in inducing T-iPSCs to some target brain regions in the forebrain, midbrain and spinal cord more efficiently than previously reported. In the future, the region-specific neuronal or glial progenitor cells induced using T-iPSCs with the developed methods will help perform reliable drug discovery and toxicity study.

Keywords: Neural differentiation, Forebrain, Spinal cord

Poster: 668

INDUCED PLURIPOTENT STEM CELL-DERIVED CEREBRAL ORGANOID MODELS OF FTD AND CADASIL REVEAL EARLY NEURONAL DEFECTS

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Dementia is a neurological syndrome characterised by the chronic, progressive loss of cognitive ability. In Frontotemporal Dementia (FTD), the symptoms are caused by degeneration of the frontal and temporal lobes of the brain, whereas in Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), recurrent small strokes eventually culminate in dementia. Due to the limitation in accessing human brain tissue, the generation of human cerebral organoids provides a useful tool in modelling neurological diseases. To model the neurodevelopmental patterns of FTD and CADASIL dementia, cerebral organoids were differentiated from patient-derived induced pluripotent stem cells (iPSCs) following a protocol established by Lancaster et al. Cerebral organoids were kept in culture for 30-60 days. After which, they were fixed and immunohistochemical staining for proliferating, differentiating and neuronal cells was carried out. 3D analysis of whole cerebral organoids using light-sheet microscopy was also conducted. We showed that iPSC-derived cerebral organoids demonstrated brain regionalisation and are viable models that can be used to decipher dementia pathogenesis. Specifically, the initial characterization of these cerebral organoids exhibits impaired neuronal localisation in early disease progression. In addition, cerebral organoids were observed to have abnormal neuroepithelial growth morphology characterized by unusual budding and neural outgrowth. Similarly, these organoids were observed to have abnormal neuronal development indicated by the lack of neural rosettes or neural rosettes with enlarged lumen. Light-sheet microscopy revealed asymmetrical neuronal differentiation in dementia-related cerebral organoids and unusual budding of these organoids may not be of neural lineage. These are some interesting observations of the organoids derived from dementia patients and more characterization needs to be conducted to further determine the ageing phenotype of these organoids. Therefore, stem cell-derived 3D human brain organoids are self-organizing and offer an unprecedented model with better structural and functional complexity resembling the human brain, serving as a promising tool for characterizing dementia.

Keywords: iPSC, Cerebral organoids, Modelling dementia

Poster: 669

ELUCIDATING THE ROLE OF DLC1 ISOFORM 1 IN HUMAN MOTOR NEURON DEVELOPMENT AND SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a motor neuron (MN) disease caused by loss of the ubiquitously expressed Survival Motor Neuron (SMN) spliceosome protein, resulting in selective degeneration of spinal MNs but the mechanisms underlying the specific loss of MNs remain unknown. A previous report showed that Deleted in Liver Cancer 1 (DLC1) is the most down-regulated gene in MNs derived from a SMA patient but its roles in MN development and SMA pathogenesis remain to be elucidated. Here, we detected a gradually increasing of DLC1-i1 expression level as human embryonic stem cells differentiated into MN lineage. Knockdown (KD) of DLC1-i1 led to a reduction in MN formation, axonal outgrowth and increase apoptosis, whereas overexpression of DLC1-i1 promoted MN differentiation with extensive axonal outgrowth. Importantly, SMN KD not only caused MN loss but also intron retention of DLC1-i1, resulting in downregulation of DLC1-i1 expression. We also confirmed decreased levels of DLC1-i1 in MNs derived from SMA patients' urine derived induced pluripotent stem cells compared to healthy individuals. Altogether, our results indicate that DLC1-i1 plays an important role in MN differentiation and deficiency of SMN causes selective loss of MNs partly through disruption of DLC1-i1 splicing.

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Keywords: spinal muscular atrophy, motor neuron, DLC1

Poster: 670

ESTABLISHMENT OF AN MBP-DELETION MOUSE MODEL BY CRISPR/CAS9 TECHNOLOGY

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Oligodendrocytes generate myelin sheath to wrap around the axons of the brain and spinal cord for insulation and increase electrical conduction. The nerve impulses travel 100 times

faster in myelinated axons while comparing to non-myelinated neurons. Many demyelination diseases are involved with oligodendrocyte dysfunction, such as multiple sclerosis (MS), and acute hemorrhagic leukoencephalitis (AHL). Besides, some neurodegenerative diseases such as cerebral palsy, Parkinson's disease, Huntingtin's disease, and schizophrenia are also oligodendroglia diseases. Therefore, glial cells hold great promise for the treatment of neurodegenerative diseases and demyelination diseases. Currently, there are about 115 clinical trials are examining the efficacy of glial cell-related therapy (www.clinicaltrials.gov). The market of MS estimate to be 40,000,000,000 USD in 2022. For the application of cell therapy, it is well known that the oligodendrocyte lineage cells (OLGs) can differentiate from embryonic stem cells (ES cells) or induced pluripotent stem cells (iPSCs), but have the risk of teratoma and insertion mutagenesis. Only mouse or rat OLGs, but not human, can be derived from fibroblasts with transcription factor induction. Until now, no protocol has been derived human-induced OLGs using chemical cocktail yet. We previously provide the very first method to generate human induced oligodendrocyte lineage cells from skin fibroblasts by small molecules. It is the fastreprogramming protocol to convert fibroblasts into oligodendrocytes, which have high efficiency and avoids the insertion of viral genes. To test the induced OLGs if rescue the demyelination of mice, this study successfully generated a myelin basic protein (MBP) deficient line by advanced severe immunodeficiency (ASID) mouse using the CRISPR/Cas9 system. We accurately knock out the large fragments of MBP sequences in the genome and successfully generate MBP-/-/ASID, which has trembling performance as natural shiverer mice. Comparing to the shiverer mice, mice with NOD background have good immunodeficiency to cell injection experiments. We expect the mice can provide a suitable animal experimental line for demyelination diseases in the future.

Funding Source: S&T development programs, executive Yuan; MOST

Keywords: oligodendrocyte lineage cells, myelin basic protein deficient mice, CRISPR/Cas9

Poster: 671

RAT CRANIAL BONE-DERIVED MESENCHYMAL STEM CELL TRANSPLANTATION PROMOTES FUNCTIONAL RECOVERY IN SPINAL CORD INJURY MODEL RATS

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The functional disorders caused by central nervous system (CNS) diseases, such as spinal cord injury, are clinically incurable and current treatments have limited effects. Previous studies suggested the therapeutic effects of cell-based therapy using mesenchymal stem cells (MSCs) for spinal cord injury. In addition, the characteristics of MSCs may depend on their derived tissues. Among the derived tissues of MSCs, we have focused on cranial bones originating from the neural crest. We previously demonstrated the therapeutic effects of cranial bone-derived MSCs (cMSCs) transplantation in ischemic stroke model rats. Therefore, cMSCs are expected to be applied for various CNS disorder models. In the present study, we aimed to demonstrate the therapeutic effects of transplantation with rat cMSCs (rcMSCs) in spinal cord injury (SCI) model rats. As the results, in vivo study, SCI model rats in rcMSC transplantation group showed better functional recovery (behavioral and electrophysiological evaluation) than those in the control and rat bone marrow-derived MSCs (rbMSCs) transplantation groups. Histological evaluation also showed a significant reduction of the cavity rate of the spine in rcMSC transplantation group. Furthermore, inflammatory related factor expressions were significantly suppressed in rat spinal cord of the rcMSCs transplantation group. In an in vitro study, the mRNA expression of brain-derived neurotrophic factor, glial cell-derived neurotrophic factor and vascular endothelial growth factor was significantly stronger in rcMSCs than in rbMSCs. In addition, conditioned medium of rcMSCs more improved the survival rate of the neural cells that exposed to inflammation or oxidative stress than that with normal medium and with conditioned medium of rbMSCs. These results suggest that cMSCs lead functional recovery through anti-inflammatory or apoptosis effects by neurotrophic factors, and cMSCs have potential as an outstanding candidate cell-based therapy for SCI.

Funding Source: This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS KAKENHI grant number 18K16561)

Keywords: Spinal cord injury, mesenchymal stem cell, cranial bone

Poster: 672

PATHOPHYSIOLOGICAL MODEL USING IPSC-DERIVED CORTICAL NEURONS FROM FTD PATIENTS WITH C9ORF72 REPEAT EXPANSION

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The C9ORF72 hexanucleotide repeat expansion is the most common genetic cause of familial frontotemporal dementia (FTD), which mainly affects the frontal and temporal lobes of the cerebral cortex. Some studies have recently suggested that one of the major pathomechanisms underlying FTD is the generation of toxic dipeptide repeat (DPR) proteins produced by C9ORF72 expansion. However, DPR-driven pathogenesis of FTD has not been fully verified in human neural cell models. While the development of human induced pluripotent stem cell (iPSC) technology has enabled the generation of patient-derived neural cells in a dish, there are no reports on iPSC-based modeling of C9ORF72-mediated FTD. In this study, we aimed to generate pathophysiological disease models using iPSCs-derived cortical neurons from FTD patients with C9ORF72 repeat expansion. First, we succeeded in generating frontal lobe-specific neurons from patient-derived iPSCs by modulating Wnt and FGF8 signaling pathway. Gene expression patterns of generated neurons were closely similar to those of human embryonic frontal lobes. Next, we found that p62 protein, which associated with autophagy, were accumulated in frontal lobe-

specific neurons derived from FTD patient iPSCs, whereas such phenotypes were not detected in neurons with other brain region identities than the frontal lobe, suggesting that the frontal lobe-specific phenotypes of FTD could be recapitulated in our culture system. p62 accumulation was also observed when DPR proteins were overexpressed in neurons from healthy control iPSCs, which indicates that DPR protein toxicity would primarily underlie the FTD pathomechanisms. Further studies into the DPR protein toxicity by C9ORF72 repeat expansions should be explored in this iPSC-based FTD models.

Keywords: Frontotemporal dementia (FTD), patient-derived iPSCs, C9ORF72 hexanucleotide repeat expansion

Poster: 673

DEVELOPING SILK SCAFFOLD-BASED PLATFORM TO GENERATE FUNCTIONAL AND REPRODUCIBLE HUMAN BIOENGINEERED FOREBRAIN ORGANOIDS

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Three-dimensional (3D) human brain organoids have rapidly become a widely used system to study brain development in a dish. Cultured over long periods of time, brain organoids provide a unique opportunity to model mature neuronal features including cytoarchitecture and cell-cell interactions reminiscent of human brain complexity. However, conventional 3D methodology is hampered by high variability in terms of morphology, size, and cellular composition and the presence of immature differentiation in the inner core. Therefore, we established a novel technological approach, using recombinant silk protein to create a bioengineered scaffold that arranges hPSCs in an organ-like configuration while maintaining their self-organizing property. We showed that silk scaffold sustained the homogeneous differentiation into mature neurons throughout all compartments of the organoid, avoiding spontaneous differentiation of cells towards meso-endodermal fate as occasionally observed in conventionally generated organoids. Whole-cell patch clamp recordings together with calcium imaging confirmed the presence of an intricate neuronal network of functionally active neurons. Furthermore, by using optical oxygen sensors that can be easily integrated into 3D cultures, we measured the oxygen gradients in silk bioengineered and conventional organoids. Our findings showed the remarkable property of silk scaffolds to form porous microarchitectures facilitating the delivery of oxygen, nutrients, and extrinsic patterning cues, thus creating more favourable growth and differentiation conditions.

Keywords: Organoids, Silk scaffold, Human forebrain development

Poster: 675

SINGLE CELL TRANSCRIPTOMICS CAPTURES FEATURES OF DEVELOPING AND MATURE DOPAMINE NEURONS IN HUMAN BRAIN ORGANOIDS AND REVEALS MORE PRECISE AND REPRODUCIBLE PATTERNING IN SILK-BIOENGINEERED CULTURE

Fiorenzano, Alessandro[1], Birtele, Marcella[1], Sozzi, Edoardo[1], Kajtez, Janko[1], Giacomoni, Jessica[1], Nilsson, Fredrik[1], Sharma, Yogita[1], Zhang, Yu[1], Emnéus, Jenny[2], Rylander Ottosson, Daniella[1], Storm, Petter[1], Parmar, Malin[1]

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Parkinson's disease (PD), one of the most common neurodegenerative disorders, is characterized by progressive loss of dopamine (DA) neurons in midbrain. Although the relatively focal degeneration in PD makes it a good candidate for cell-based therapies, the inaccessibility of functional human brain tissue and the inability of two-dimensional in vitro cultures to recapitulate the complexity and function of dopaminergic circuitries have made the study of human midbrain functions and dysfunctions challenging. Three-dimensional (3D) neural culture systems provide an opportunity to understand complex organization in a physiologically relevant cellular context and to better obtain functional maturity of neurons in vitro. In this study, we designed a new protocol for differentiating human pluripotent stem cells (hPSCs) into 3D human regionalized organoids that, when patterned towards a ventral midbrain (VM) fate, results in the formation of authentic and functional DA neurons. By combing CRISPR-Cas9 gene editing with transcriptional profiling at single-cell resolution, we showed that tyrosine hydroxylase neurons exhibit molecular and electrophysiological properties of mature DA neurons, expressing functional receptors of A9 and A10 neurons as well as being able to release dopamine. However, the use of a conventional 3D methodology resulted in high variability in terms of morphology, cellular composition, and inability to fully recapitulate late and functionally mature stages of human brain development. We therefore established a novel technological approach using recombinant silk protein functionalized with a cell-binding fibronectin motif to create a bioengineered scaffold that arranges hPSCs in an organ-like configuration while maintaining their self-organizing property. The silk microscale internal scaffold generates cavities within organoids that enable the delivery of oxygen and nutrients, maintaining health of cells and preventing necrosis regardless of their size and geometric shape. In contrast to traditional 3D culture systems, bioengineered silk organoids supports highly reproducible DA organoid differentiation, leading to long-term expansion of subtype specific VM DA neurons that are transcriptionally and functionally similar to human fetal DA neurons.

Keywords: PLURIPOTENT STEM CELL DIFFERENTIATION, VENTRAL MIDBRAIN ORGANOID DIFFERENTIATION, DOPAMINE NEURON DIVERSITY

Poster: 676

UNRAVELLING THE ROLE OF THE CILIARY PROTEIN CEP290 IN IPSC-DERIVED HUMAN CORTICAL NEURONS

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Ciliopathies are heterogeneous Mendelian disorders caused by dysfunction of primary cilia, ubiquitous organelles crucial for signal transduction during development and cell homeostasis. Patients suffer from phenotypes affecting multiple organs, with frequent central nervous system involvement. CNS disease spectrum ranges from intellectual disability without morphological anomalies, variable seizure activity, to hindbrain malformations, as seen in the iconic ciliopathy Joubert syndrome. To date, the role of primary cilia in human cortical neurons has been little investigated, mainly because of their limited accessibility. Using CRISPR editing, we generated knockout induced pluripotent stem cell lines for the ciliary gene CEP290, followed by neural differentiation. As ciliary protein composition is cell-type dependent, we characterized the CEP290 interactome in neural stem cells (NSCs) through immunoprecipitation followed by mass spectrophotometry, identifying an interaction with APC and β-catenin, two proteins involved in Wnt signaling and in NSC maintenance. To understand the cellular mechanisms implicating CEP290 during neural development, we studied the consequences of CEP290 knockout on neural patterning, ciliation and signalling by western blot and immunochemistry. CEP290 edited lines can differentiate in vitro with no major difference of ciliary length and number. Neural differentiation of the mutant cell lines generated a higher propensity of FOXG1 expressing NSCs compared to controls, a difference that was corrected by inhibiting Wnt signaling in the control line in the first days of differentiation. These results suggest a differential baseline level of Wnt activation of the mutant cells, a hypothesis that is currently under investigation with quantification of Wnt target gene expression levels. Our model suggests a new pathophysiological mechanism with a role for the ciliary protein CEP290 in neural differentiation through involvement of Wnt signaling.

Funding Source: Praeclare Clinical Research Priority Program, Swiss national science foundation

Keywords: wnt signaling, primary cilia, neural stem cells

Poster: 677

EFFECTS OF THE ORGANOPHOSPHATE VX ON IPSC-DERIVED NEURONS: GENETIC, PROTEIN AND MORPHOLOGICAL ANALYSIS

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The need for a human neuro-muscular test model to find new potential nerve agent antidotes is highlighted by the increasing global political uncertainty, such as the use of nerve agents especially in Syria in recent years. By the generation and differentiation of human induced pluripotent stem cells (hiPSCs) into neurons, in particular motoneurons, a high throughput screening is made possible. Thus avoiding the use of ethically highly discussed embryonic stem cells. For the generation of hiPSCs, the pluripotency-inducing Yamanaka factors (Oct3/4, Sox2, c-Myc, Klf4) were inserted into human fibroblasts by plasmid nucleotransfection. During their differentiation into neurons continuous morphological documentation, identification and characterization by means of immunohistochemical staining, PCR and flow cytometry was performed. The derived neurons were exposed to 400 µM, 600 µM, 800 µM and 1 mM VX and were incubated for 5 days. During incubation time, morphologic changes were captured by the IncuCyte, a real-time imaging tool. Subsequently the cells were collected and analyzed by PCR and Western blot. The neuronal precursor structures showed a positive staining for PAX6 and ß3-Tubulin. In the further course of cultivation Synapsin- and Peripherin-positive neuronal networks evolved which could also be identified as motoneurons by staining with MNX, Islet-1 and SMI-32. During exposure to VX a loss of neurites and a general cell shrinkage was observed. PCR-Analysis showed that apoptosis-related genes as BAD, CASP5, CASP10 and BIK were upregulated. Proteins involved in apoptosis execution as caspase-10 and HSP60 were detected by Western blot analysis. These results suggest that the intrinsic pathway as well as the extrinsic pathway of apoptosis are activated after VX exposure. Further investigations, e.g. on the expression of caspase 3, caspase 8 and caspase 9, are necessary for clarification. This will provide new insights into the pathomechanism of nerve agents at the molecular level and might help to identify new therapeutic approaches.

Keywords: iPSC-derived Neurons, Organophosphate, Apoptosis

Poster: 678

DIRECT CONVERSION OF HUMAN GLIA AS A NOVEL ROUTE FOR BRAIN REPAIR

Giacomoni, Jessica, Bruzelius, Andreas, Habekost, Mette, Mattsson, Bengt, Ottosson, Daniella Rylander, Fiorenzano, Alessandro, Parmar, Malin

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Parkinson's disease (PD), one of the most common neurodegenerative disorders, is primarily characterized 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 within the brain parenchyma. Proof-of concept that in vivo conversion can be a viable future option has been provided in rodent studies. However, the inaccessibility of human brain tissue and the lack of an in vitro culture system that recapitulates the complexity of the human brain represent major limitations in the development and pre-clinical validation of such approach. We have 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 are currently establishing a 3D culture model that more closely recapitulates the in vivo environment and are evaluating it as a pre-clinical model of human glia conversion. Preliminary

data indicate that our approach increases conversion efficiency, accelerates the reprogramming process compared to the 2D culture system and results in mature neuronal phenotypes with electrophysiological function within 3 weeks. Furthermore, reprogramming in 3D allows long term cultures and is applicable for a variety of neuronal conversion protocols.

Keywords: neuronal reprogramming, spheroid model, hESC

Poster: 679

MODELING SENSORY NEUROGENESIS WITH HUMAN PLURIPOTENT STEM CELL DERIVATIVES IN VITRO AND IN CHICK EMBRYO

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The spatiotemporal structure of cell fate decisions in the vertebrate peripheral nervous system (PNS) has been intensively studied in chick and mouse embryos. Several transitory embryonic ectodermal tissues are at the origin of sensory neurons; the earliest is the neural plate border (NPB), which transforms into the neural crest (NC) and preplacode (PP) in the head region (cranial), and into the NC in the trunk region. The PNS development in human embryos was less investigated and lacked dynamic approaches. To address this gap, we aimed to model the initial stages of sensory neurogenesis by following the human pluripotent stem cell (hPSC) differentiation. We employed several treatments with BMP and WNT pathway agonists and/or antagonists, and investigated the differentiation of hPSCs toward NPB, NC, PP, neural plate and non-neural progenitors. We monitored cell morphology, expression of cell surface markers and expression of evolutionary conserved transcription factors, which allowed to define combinatorial molecular codes for NPB, cranial/trunk premigratory NC (PNC), cranial/trunk migratory NC (MNC) and PP stages. We further applied Notch inhibition and found that several precursor cells differentiated into sensory neurons co-expressing BRN3a and ISL1; the PNC and PP progenitors generated ~90% sensory neurons in 5 days. We transplanted different GFP-labeled human sensory precursors and post-mitotic neurons into in cranial and trunk regions of chick embryos at developmental stages corresponding from 5 to 25 somite pairs. The histological investigation at day 4-6 post-transplantation showed epithelial-like aggregates formed by progenitor cells in both cranial and trunk regions, without neuronal differentiation. By contrary, transplanted early post-mitotic neurons (both PP and cranial PNC-derived) integrated and differentiated within the forming chick cranial sensory ganglia, especially in the trigeminal ganglia. However, in our time-frame, the trunk-like NC-derived neurons did not integrate into the chick dorsal root ganglia. This model allows to identify the cell signatures and trajectories corresponding to human cranial sensory development and to align them with the sensory neurogenesis and differentiation in chick embryo.

Funding Source: This work was supported by Austrian Science Fund (FWF), Project P26886-B19, Austria.

Keywords: peripheral sensory neurons, human neural crest development, human preplacode development

Poster: 680

RAPID FORWARD PROGRAMMING OF HUMAN IPS CELLS INTO SENSORY NEURONS

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Disruption of sensory neuron and particularly nociceptor function is associated with a number of clinical disorders. One in five people in Europe suffer from chronic pain, and a majority of patients report that their medical treatment does not meet their expectations. Human iPSC-derived sensory neurons provide perspectives for investigating pathomechanisms and assessing potential therapies directly in disease-relevant cells. Recent progress in transcription factor-based cell programming may further promote this approach. We engineered iPSCs to express a combination of two (NGN1, BRN3A; 'NB') or three transcription factors (NGN1, BRN3A, ISLET1; 'NBI') from a safe harbor locus in an inducible manner. Upon 7 days of transgene induction both combinations yielded sensory neurons expressing key markers such as PRPH, TRPV1 and NAV1.7. Compared to NB, NBI induced an earlier upregulation of PRPH and enhanced expression of the nociceptor-specific gene TRPV1. Furthermore, NBI robustly induced pure neuronal cultures independent of the iPSC seeding density. Exposure to pain specific stimuli αβ-ATP (P2X3) and Capsaicin (TRPV1) significantly increased neuronal firing rate, validating nociceptor-specific functionality in these neurons. Finally we employed iPSCs from a chronic pain patient suffering from inherited erythromelalgia (IEM) for disease modeling. IEM is characterized by extreme burning pain and is attributed to gain-of-function mutations in the SCN9A gene coding for the peripheral sodium channel NAV1.7. Patient-derived NBI neurons showed an increased firing rate in multi-electrode array assays recapitulating changes in cellular functionality that could be associated with the sensation of chronic pain in affected individuals. Taken together, we here present a forward programming approach for the fast and efficient generation of pure and functional human sensory neurons suitable for disease modeling.

Keywords: Forward Programming, Sensory Neuron, Pain

Poster: 681

IMPAIRED AUTOPHAGY IN FTD3 CHMP2B CAUSES DISTORTED ENERGY METABOLISM AND LEADING TO REACTIVE ASTROCYTE PHENOTYPES

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Frontotemporal dementia type 3 (FTD3) is a neurodegenerative disorder caused by a truncating mutation in charged multivesicular body protein 2B (CHMP2B). The protein CHMP2B is a part of the ESCRTIII complex necessary for autophagy, endosomal trafficking and protein degradation. FTD3 previously reported to cause endosomal-lysosomal fusion problems in neurons. To study the role of astrocytes in FTD3, we established a disease model using human induced pluripotent stem cells (hiPSCs) from patients carrying the mutation in CHMP2B and isogenic controls generated via the CRISPR/Cas9 system. In

addition, we also generated heterozygous and homozygous CHMP2B-mutant hiPSC via CRISPR/Cas9 knock-ins to study mutation specific changes in a different unrelated genetic background. Finally, these findings were validated in CHMP2B mutant mouse. With these model, CHMP2B mutant astrocytes displayed FTD3 pathology by exhibiting imbalance autophagy with increased P62 and LC3 expression levels accompanied by accumulation of autophagosomes, which is in correspondence to our previous observation in CHMP2B mutant neurons. In support to these findings, we also observed hampered mitochondrial dynamics such as elongated mitochondrial morphology and increased reactive oxygen species (ROS) levels, resulting in impaired mitophagy. We postulate activation of autophagic mechanisms affects the mitochondrial turnover and cause hypometabolism thereby affecting the normal homeostasis of the resting astrocytes to become reactive astrocytes by releasing toxic cytokines. Collectively our overall findings illustrate that distorted astrocyte triggers a series of gene expression and protein changes which activated an intracellular reactivity response through NF-κB pathway which resulted in astrogliosis expression and thereby severely disrupting healthy neurons. Rescue experiments targeting ROS with ursodeoxycholic acid could restore ROS levels back to normal levels, indicating that the failed removal of abnormal mitochondria triggers the pathological cascade in CHMP2B mutant astrocytes culminating in the formation of neurotoxic reactive astrocytes. Overall, our model displays how CHMP2B mutant astrocytes contribute to neurodegeneration and disease progression in FTD3.

Funding Source: Independent Research Fund Denmark (FTP, 109799), Innovation Fund Denmark (BrainStem, 4108-00008B & NeuroStem, 4096-00001B), Alzheimer Foundation Denmark, Novo Nordisk Foundation (GliAD—NNF18OC0052369 & NNF19OC0058399).

Keywords: CHMP2B-FTD3 astrocytes, hiPSC-derived astrocytes, autophagy

Poster: 682

IN VITRO EFFECT OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS CONDITIONED MEDIUM ON THE OXIDATIVE STRESS INDUCED-CELLULAR SENESCENCE OF NORMAL HUMAN DERMAL FIBROBLAST

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Cellular aging is first described as the progressive and irreversible loss of proliferative potential and functional capacity of cells. Fibroblasts are one of the most widely used cells in the study of oxidative stress induced-cellular senescence and replicative cellular senescence. Mesenchymal stem cells (MSC) are multipotent cells that can be derived from different organs and tissues such as bone marrow, adipose tissues, umbilical cord blood and dental pulp tissues. MSC are able to secrete bioactive factors in the medium and the conditioned medium acts as chemoattractant for cell repair. However, very few studies have been done to explore the anti-aging features of MSC in human cells. Thus, the effect of MSC conditioned medium on cellular senescence of normal human dermal fibroblast (NHDF) was investigated in this study. NHDF was first treated with 200 µM hydrogen peroxide (H2O2) for 2 hours and allowed to recover for 3, 5 and 7 days to develop the senescent model. Treated NHDF showed senescent-like features in expression level of senescent gene and presence of beta-galactosidase enzyme. Senescent NHDF was further exposed to umbilical cord-derived mesenchymal stem cells (UC-MSC) conditioned medium for 24 hours and 48 hours. UC-MSC conditioned medium is expected to ameliorate the senescent features in treated NHDF. This suggested that MSC are able to secrete bioactive factors that can exert anti-aging properties in its culture medium.

Funding Source: This research project is funded by UTAR Research Fund (UTARRF).

Keywords: Normal Human Dermal Fibroblast (NHDF), Cellular Aging, Umbilical cord-derived Mesenchymal Stem Cell

12:00 - 13:00 EDT

POSTER SESSION 7

CELLULAR IDENTITY

Poster: 516

POTENTIAL ROLE OF G-PROTEIN-COUPLED RECEPTOR SIGNALLING IN GLIA-TO-NEURON CONVERSION IN THE POSTNATAL MOUSE CEREBRAL CORTEX

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The identity of glial cells residing in the brain can be reprogrammed into induced neurons by forced expression of neurogenic transcription factors (TFs) (Heinrich et al. 2014, Gascón et al, 2016)). We have targeted populations of proliferative glial cells of the mouse postnatal cortex by retroviruses (RVs) encoding for reprogramming factors, e.g. proneural genes in combination with Bcl2. Forced expression of Neurog2 and Bcl2 yields in high glia-to-neuron conversion efficiencies. Importantly, the proliferative state of iNs prior to reprogramming is confirmed with EdU incorporation. Nevertheless, glia-derived iNs remain immature in terms of morphology and functional properties. Thus, one major goal aims at improving iN maturation. Functional maturation of iNs has been improved by the co-expression of neurogenic TFs with excitatory DREADDs, the receptor hM3Dq, which, after its activation by clozapine-N-oxide (CNO) activates a Gq-protein coupled receptors (GPCRs) signalling pathway resulting in increased excitability of iNs. In this project, we aim at studying if the activation of endogenous GPCRs systems affects the maturation of iNs. Given the well-known role of dopamine in the maturation and modulation of newly generated cortical neurons during development (Stanwood et al., 2005, Wang et al., 2009; Berlanga et al., 2011)), here we aim at inducing glia-to-neuron conversion in the postnatal cerebral cortex of P5 mice in which dopaminergic neurons express the receptor hM3Dq. In this model, we perform immunohistochemical, morphological and functional analyses to reveal the degree of iN maturation upon dopaminergic activation. In addition, we are currently performing in situ hybridization assays (i.e. RNAscope) in combination with immunohistochemistry to uncover whether

iNs express dopamine receptors. Altogether, these experiments will allow us to assess the effect of dopamine signalling on the maturation and integration of glia-derived iNs.

Funding Source: This work was supported by a grant from the Wellcome Trust (206410/Z/17/Z)

Keywords: Neuronal reprogramming, Regeneration, Immunohistochemistry

Poster: 575

THERAPEUTIC REDUCTION OF PIKFYVE AMELIORATES NEURODEGENERATION AND IMPROVES MOTOR FUNCTION BY INDUCTION OF SECRETORY AUTOPHAGY

Linares, Gabriel R.[1], Hung, Shu-Ting[1], Chang, Wen-Hsuan[2], Hong, Sarah[2], Lee, Emily[2], Mendoca, Stacee[2], Santana, Manuel[1], Eoh, Yunsun[1], Ichida, Justin[1]

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Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease characterized by the degeneration of motor neurons. In a previous unbiased phenotypic screen to identify compounds that rescue the survival of induced motor neurons (iMNs) generated from iPSCs derived from multiple ALS patients with known or unknown causal mutations, we found that PIKFYVE kinase was a broadly efficacious target. PIKFYVE is an endosomal lipid kinase that normally promotes autophagy. Surprisingly, inhibition of PIKFYVE rescues neurodegeneration mechanistically by blocking autophagosome-lysosome fusion, which induces secretory autophagy to robustly clear misfolded proteins such as TDP-43 that normally accumulate in the cytoplasm and drive neurodegenerative processes. Antisense-mediated suppression of PIKFYVE targeted to the central nervous system rescued motor deficits and extended lifespan in a TDP-43 transgenic mouse model of ALS. Notably, the protective action of PIKFYVE inhibition was abolished by treatment with GW 4869, a known inhibitor of exosomal secretion. In addition, we provide evidence for PIKFYVE target engagement in vivo by increased CSF levels of optineurin following PIKFYVE inhibition. Moreover, the genetic reduction of PIKFYVE in a haploid deficient mouse crossed with a TDP-43 transgenic mouse also showed beneficial effects in the mutant mice, thus confirming the utility of this target in vivo. Histological analyses demonstrated that the reduction of PIKFYVE significantly decreased pathological levels of pTDP-43 and abrogated neuronal loss in in the spinal cord of mutant TDP-43 transgenic mice compared to negative control ASO treated TDP-43 transgenic mice. Our results demonstrate that PIKFYVE kinase suppression and the activation of secretory autophagy are promising therapeutic strategies to broadly rescue ALS motor neuron degeneration.

Funding Source: 1R01NS097850-01

Keywords: PIKFYVE, TDP-43, secretory autophagy

Poster: 701

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

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The ability to generate induced pluripotent stem cells (iPSCs) from differentiated somatic cells via ectopic expression of OCT4, KLF4, SOX2, and MYC (OKSM) has enabled engineering of 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 maintaining pluripotency during development. Finally, we show preliminary evidence that cells can be converted from unprimed states to primed states with the use of reprogramming boosters to increase overall reprogramming efficiency. This work is poised to answer longstanding questions about the existence and nature of rare cells primed for reprogramming. More broadly, it will help us identify new pathways to modulate reprogramming in predictable ways and reveal the molecular basis of plasticity in seemingly differentiated cells.

Funding Source: T32GM007170 Medical Scientist Training Grant, Brass (PI), F30HD103378 National Research Service Award, Jain (PI)

Keywords: reprogramming, iPSC, barcoding

Poster: 702

INO80 PROMOTES H2A.Z OCCUPANCY TO REGULATE CELL FATE TRANSITION IN PLURIPOTENT STEM CELLS

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The INO80 chromatin remodeler is involved in many chromatin-dependent cellular functions. However, its role in pluripotency and cell fate transition is not fully defined. We examined the impact of Ino80 deletion in the naïve and primed pluripotent stem cells. We found that Ino80 deletion had minimal effect on self-renewal and gene expression in the naïve state, but led to cellular differentiation and de-repression of developmental genes in the transition toward and maintenance of the primed state. Mechanistically, INO80 pre-marked gene promoters that would adopt the H3K4me3 and H3K27me3 bivalent histone modifications. It promoted H2A.Z occupancy at these future bivalent domains to facilitate H3K27me3 installation and maintenance as well as downstream gene repression. Thus, INO80-dependent H2A.Z occupancy is a critical licensing step for bivalency and poised gene expression in pluripotent stem cells. Our results uncovered an unexpected function of INO80 in H2A.Z deposition and gene repression, and an epigenetic mechanism by which chromatin remodeling, histone variant and modification coordinately control cell fate.

Funding Source: National Institutes of Health

Keywords: INO80, Bivalency, Pluripotent stem cells

Poster: 703

CTCF IS A BARRIER FOR TOTIPOTENT-LIKE REPROGRAMMING

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Totipotent cells have the ability of generating embryonic and extra-embryonic tissues. Interestingly, a rare population of cells with totipotent-like potential was identified within ESC cultures. These cells, known as 2 cell (2C)-like cells, arise from ESC and display similar features to those found in the totipotent 2 cell embryo. However, the molecular determinants of 2C-like conversion have not been completely elucidated. Here, we show that CTCF is a barrier for 2C-like reprogramming. Indeed, forced conversion to a 2C-like state by DUX expression was associated with DNA damage at a subset of CTCF binding sites. Endogenous or DUX-induced 2C-like ESC showed decreased CTCF enrichment at known binding sites, suggesting that acquisition of a totipotent-like state is associated with a highly dynamic chromatin architecture. Accordingly, depletion of CTCF in ESC efficiently promoted spontaneous and asynchronous conversion to a totipotent-like state. This phenotypic reprogramming was reversible upon restoration of CTCF levels. Furthermore, we showed that transcriptional activation of the ZSCAN4 cluster was necessary for successful 2C-like reprogramming. In summary, we revealed the intimate relation between CTCF and totipotent-like reprogramming.

Funding Source: Intramural Research Program (NIH)

Keywords: CTCF, totipotent-like cells, ZSCAN4

Poster: 704

SYNTHETIC GENOMIC RECONSTITUTION REVEALS PRINCIPLES OF MAMMALIAN HOX CLUSTER REGULATION

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Precise Hox gene transcription is crucial for embryonic patterning. Local transcription factor binding, dynamic chromatin domains, and distal enhancer elements have emerged as major regulatory modes controlling Hox gene expression initiation and maintenance. However, quantifying their relative contributions has remained elusive. Here, we introduce a novel conceptual framework for the study of gene regulation termed 'synthetic regulatory reconstitution' and apply it to the mammalian HoxA cluster. To measure HoxA intrinsic regulatory potential and distal enhancer contributions, we de novo synthesized and delivered variant rat HoxA clusters (130-170 kilobases) to an ectopic location in the mouse genome. We find that a minimal HoxA cluster induces the appropriate gene set and establishes distinct chromatin domains in response to patterning signals. The ectopic HoxA cluster requires distal enhancers for full transcriptional output. These results suggest that the genomically compact Hox clusters contain all the information to decode patterning signals and maintain positional information.

Funding Source: R01NS100897 C32560GG RM1HG009491

Keywords: Hox, Genomic engineering, epigenetic

Poster: 705

A NOVEL ROAD MAP OF REGULATORY SMALL RNAS DURING SOMATIC CELL REPROGRAMMING TO PLURIPOTENCY

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Cell fate decisions during lineage differentiation and reprogramming relies on signaling pathways as well as transcriptional and posttranscriptional gene regulatory loops. Small non-coding RNAs (scnRNAs) are key players in finetuning

gene expression with microRNAs being arguably the best characterized class in sncRNA biogenesis and effector functions. Remarkably, the repertoire sncRNAs has been expanding since the discovery of the RNA interference pathways and the development of high throughput sequencing technologies. For example, tRNA-derived small RNAs (tsRNAs) and rRNA-derived small RNAs (rsRNAs) are emerging as new players in regulating gene expression. However, the diversity and function of these scnRNAs during re-establishment of pluripotency and lineage differentiation remain unexplored. Here we use Panoramic RNA Display by Overcoming RNA Modification Aborted Sequencing (PANDORA-seq) as a novel small RNA sequencing technology to profile scnRNAs during transcription factor mediated reprogramming of mouse embryonic fibroblasts to induced pluripotent cells. Our approach uncovers sncRNAs during reprogramming at an unprecedented depth and reveals a novel dynamic landscape. We find that the majority of tsRNAs and rsRNAs are downregulated during the reprogramming process. Functional analysis of select candidates during embryonic stem cell differentiation reveals their impact on lineage specific transcriptional programs possibly through their effect on cellular translation. Together, our new method to clone small RNAs allowed us to systematically characterize sncRNAs during reprogramming and identify candidates involved in cell fate determination.

Keywords: Small non-coding RNAs (scnRNAs), Reprogramming, Pluripotency

Poster: 706

CHARACTERIZING X CHROMOSOME STRUCTURE IN NAÏVE AND PRIMED HUMAN PLURIPOTENT STEM CELLS USING 3D GENOMICS APPROACHES

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Female human pluripotent stem cells (hPSCs) are highly desirable models for studying X chromosome inactivation (XCI), as both pluripotency and XCI play out during peri-implantation embryological development. Recent studies incorporating single-cell RNA sequencing have demonstrated that transcript-based technologies are insufficiently developed to unequivocally resolve XCI dynamics in hPSCs. Alternatively, 3D genomics holds immense promise for studying XCI by characterizing changes to the 3D conformation of the active and inactive X chromosomes. We therefore sought to bypass the ongoing issues with transcript-based methods and characterized the 3D state of the X chromosome in female naïve and primed hPSCs. Through a combination of Hi-C and DNA MERFISH, we analyzed 40 topologically-associated domains (TADs) across the X chromosome in multiple naïve and primed hPSC lines. Imaging data was subsequently used to reconstruct the 3D conformation of the TADs in the X chromosomes in each cell state. From this, we determined that naïve cells typically exhibit 3D conformational signatures of active X chromosomes. Naïve X chromosomes generally compartmentalize about the centromere and appear more disperse than primed X chromosomes on average. Through unsupervised clustering analysis, we were unable to find any strong evidence of subpopulations within the 3D structural variation of naive X chromosomes; naive hPSCs therefore likely contain 2 active X chromosomes that all cluster into one population. On the other hand, hPSCs in the primed state exhibit much more compact X chromosomes. In some lines, we were able to detect superdomain compartmentalization about the DXZ4 locus, which is a canonical signature of the inactive X chromosome's 3D structure. In all, we characterized the general 3D structure of the X chromosome in female naive and primed hPSCs. Our results demonstrate the utility of 3D genomics methods in probing XCI regulation in hPSCs, the characterization of which has proven quite difficult through transcript-based methods.

Funding Source: Research reported in this study was supported by Yale University. B.P. was supported by NIH T32 GM007499 and the Lo Graduate Fellowship for Excellence in Stem Cell Research.

Keywords: X chromosome inactivation, 3D genomics, Peri-implantation embryo

Poster: 707

THE ANNA KARENINA MODEL OF BETA CELL MATURATION IN DEVELOPMENT AND THEIR DEDIFFERENTIATION IN TYPE 1 AND TYPE 2 DIABETES

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Loss of mature β cell function and identity, or β cell dedifferentiation, is seen in all types of diabetes mellitus. Two competing models explain β cell dedifferentiation in diabetes. In the first model, β cells dedifferentiate in the reverse order of their developmental ontogeny. This model predicts that dedifferentiated β cells resemble β cell progenitors. In the second model, β cell dedifferentiation depends on the type of diabetogenic stress. This model, which we call the "Anna Karenina" model, predicts that in each type of diabetes, β cells dedifferentiate in their own way, depending on how their mature identity is disrupted by any particular diabetogenic stress. We directly tested the two models using a β cell-specific lineage-tracing system coupled with RNA-sequencing in mice. We constructed a multidimensional map of β cell transcriptional trajectories during the normal course of β cell postnatal development and during their dedifferentiation in models of both type 1 diabetes (NOD) and type 2 diabetes (BTBR-Lepob/ob). Using this unbiased approach, we show here that despite some similarities between immature and dedifferentiated β cells, β cells dedifferentiation in the two mouse models is not a reversal of developmental ontogeny and is different between different types of diabetes.

Keywords: β cell dedifferentiation, β cell development, β cell identity

Poster: 708

THE MOLECULAR MECHANISM OF H3K9ME3-HETEROCHROMATIN LOSS ON PROTEIN-CODING GENES DURING EMBRYONIC DEVELOPMENT

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Gene silencing by chromatin compaction is integral to establishing and maintaining cell fates. The trimethylated histone 3 lysine 9 (H3K9me3) modification is known to be involved in the gene silencing, and the level of H3K9me3 is reduced in embryonic stem cells compared to differentiated cells, suggesting that the modification dynamically changes during development. We previously developed a new method to map compacted heterochromatin from low-cell number samples during mouse embryo development. We discovered a large number of silent, lineage-specific genes embedded in H3K9me3-decorated heterochromatin in undifferentiated endoderm cells and loss of H3K9me3 at such genes during hepatocyte and beta-cell differentiation, concomitant with cell type-specific expression (Dario et al., Science 2019). However, how the loss of H3K9me3 specifically occurs at lineage-specific genes remains unknown. To uncover the molecular mechanism of loss of H3K9me3 during development, we hypothesized that lineage-specific transcription factors specifically recruit H3K9me3 demethylases (KDM4s) to specific locus during development. To test this hypothesis, we identified functional regulatory elements around hepatocyte-specific genes that exhibit loss of H3K9me3 during liver development. By focusing on the enhancer region in each developmental stage, we found the tendency that loss of H3K9me3 around liver-specific active enhancers is observed prior to differentiation into the specific cell type of liver cells. By focusing on the enhancers that selectively lose H3K9me3 in development, we identified candidate TF motifs that are important for KDM4s recruitment. Now we are trying to test the importance of the candidate TFs for KDM4s recruitment around hepatocyte-specific genes by using the protein extract from mice liver. Also, we are trying to test the effect of the knockdown of these TFs for loss of H3K9me3 by using in vitro liver differentiation system. The combination of the approaches should unveil how H3K9me3-heterochromatin loss enables tissue-specific embryonic development.

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Keywords: H3K9me3, KDM4s, hepatocyte/beta-cell differentiation

Poster: 710

SEQUENTIAL DYNAMICS OF TRANSCRIPTION FACTORS SOX2 AND NANOG DURING HUMAN EMBRYONIC STEM CELL DIFFERENTIATION AND REPROGRAMMING

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Human induced pluripotent stem cells (hiPSCs) hold enormous promise as a personalized therapy for treating a variety of human diseases. There are well-established methodologies to induce pluripotency in different types of patient cells and to differentiate hiPSCs toward various cell fates. However, the exact cellular mechanisms that govern entering and exiting the pluripotency state remain poorly understood. To address these challenges, I investigated the temporal dynamics of pluripotency transcription factors (TFs) underlying stem cell pluripotency. I developed a novel fluorescent reporter system in the H9 human embryonic stem cell line to simultaneously monitor the single-cell dynamics of the pluripotency TFs SOX2 and NANOG. This was achieved by fluorescently tagging the SOX2 and NANOG genes with unique fluorescent proteins using CRISPR/Cas9-mediated gene editing. This live-cell reporter provides a real-time indicator of when cells enter a partially or fully pluripotent state and the sequential dynamics associated with pluripotency TF gain or loss. With this cell line, I differentiated the pluripotency reporter cell line to endothelial cells (ECs), hematopoietic progenitor cells (HPCs), lung progenitor cells (LPCs), and neural progenitor cells (NPCs). Using time-lapse fluorescence microscopy to monitor the dynamics of pluripotency loss during cell fate decisions, I found that SOX2 and NANOG show staggered timing in their loss of expression that varied according to the cell's commitment to a particular fate. Upon reprogramming of these differentiated cell types back into a pluripotent stem cell state, reactivation of SOX2 and NANOG expression followed the reverse temporal sequence. These results indicate that pluripotency TFs follow a unique sequence of expression that is specific to the cell lineage being reprogrammed. These findings provide fundamental insights into the sequential pluripotency TF dynamics of SOX2 and NANOG across multiple cell lineages during differentiation and reprogramming.

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Keywords: pluripotency, differentiation, reprogramming

Poster: 711

SINGLE-CELL DETECTION OF DIVERSE COPY NUMBER VARIATIONS IN PLURIPOTENT STEM CELLS

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Microenvironments can in principle affect genome instability and subsequent selection, and reporters and sequencing at the single cell level might help identify conditions in vivo and in vitro that initiate and propagate changes in chromosome copy numbers. Cancer genome studies have already shown frequent chromosome gains favor key oncogenes and losses favor tumor suppressors, but bulk sequencing methods and labor-intensive karyotyping are not ideal for detecting genetic changes in sub-populations, which is important for the application of pluripotent cells. To easily capture the effects of chromosomal instability that may occur in the life cycle of a single cell, we have tagged single alleles of an array of different chromosomes using CRISPR/Cas9 technology. Tagging a single allele (by inserting the expression sequence for fluorescent reporters such as GFP or RFP onto a constitutively expressed gene), we have created a fluorescent reporter system that gives us a direct readout of chromosomal loss. A single tagged allele is enough for a cell to fluoresce. Upon undergoing stressful perturbations, individual cells may lose fluorescence, which would be a fluorescent-based readout that the tagged chromosome was lost. Tagging of single alleles has allowed us to show in vivo teratomas typically lose fluorescence (indicating chromosome loss) in a sub-population. Furthermore, these cells continue demonstrate the potential to continue propagating, highlighting the link between genomic instability and the potential to generate aberrant karyotyped progeny cells. In vitro studies replicate the mitotic confinement of 3D growth and likewise show rapid loss of fluorescence and chromosomes - unlike standard 2D cultures. The fluorescence reporter approach visually confirms viability and heritability, and very deep sequencing in single cell RNA-seq provides a snapshot of losses and gains of six or more chromosomes in ~0.1 to ~5% of transcriptionally homogeneous, cycling pluripotent cells. Teratomas show the expected diversity in differentiation, and the current challenge is to relate lineage to the chromosome changes.

Funding Source: National Science Foundation Graduate Research Fellowship

Keywords: Genomic instability, iPSC, Mechanogenomics

Poster: 712

INFLATION-COLLAPSE DYNAMICS DRIVE PATTERNING AND MORPHOGENESIS IN INTESTINAL ORGANOIDS

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Intestinal organoids generate stem cell zones (SCZs) of typical size without a spatially-structured environment, making them an excellent candidate for understanding the principles of self-organization. We have introduced a method to culture organoids that improves the viability and homogeneity of intestinal organoid cultures to enable long-term time-lapse imaging of multiple organoids in parallel. We use this method to probe the factors governing stem cell zone size. We found that SCZs ex vivo are largely shaped by inflation-dependent fission events under strong control of ion channel-mediated inflation and mechanosensitive Piezo-family channels. Fission occurs through stereotyped modes of dynamic behavior that differ in their coordination of budding and differentiation. Imaging and single cell transcriptomics show that inflation drives acute stem cell differentiation and induces a stretch-responsive cell state characterized by large transcriptional changes, including upregulating Piezo1. Our results reveal an intrinsic capacity of the intestinal epithelium to self-organize by modulating and then responding to its mechanical state.

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Keywords: cell fate, organoid, single cell RNA sequencing

Poster: 713

INVESTIGATING EVOLUTIONARILY CONSERVED ENHANCERS IN THE CONTRASTING REGENERATIVE POTENTIAL OF MOUSE AND ZEBRAFISH INNER EAR HAIR CELLS

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The major cause of hearing loss is damage to the inner ear cochlear sensory hair cells. Non-mammalian vertebrates such as zebrafish can replenish sensory hair cells throughout life from the proliferation and transdifferentiation of neighboring supporting cells. In contrast, mouse cochlear supporting cells can only be reprogrammed to a hair cell-like state up to one week after birth. This indicates that mouse supporting cells become more lineage restricted compared to zebrafish supporting cells during development and maturation. Preliminary mouse data suggest that this age-related loss of supporting cell plasticity may be explained by epigenetic silencing of enhancers that regulate the expression of genes that are critical to hair cell lineage specification. As DNA sequences within enhancers have been shown to recruit repressive transcription factors or epigenetic silencing complexes, we asked the question whether mouse hair cell enhancers contain repressive elements that restrict them

from regulating proper spatiotemporal expression of hair cell-specific genes for transdifferentiation. By performing sequence alignment of putative mouse and zebrafish hair cell enhancers, I have derived a list of highly conserved hair cell enhancers between the two species. Using fluorescent reporter zebrafish models, I have shown that both mouse and zebrafish versions of the homologous enhancers exhibit similar localization of reporter activity in the zebrafish inner ear. To interrogate whether DNA elements within mouse hair cell enhancers contribute to enhancer silencing, I am comparing reporter activities of the mouse versus zebrafish hair cell enhancers in transgenic zebrafish. These results will reveal how epigenetic regulations are involved in broad tissue regeneration and how evolutionary changes in DNA sequences may explain differences seen in regeneration potential across different species.

Funding Source: NIH NIDCD 2T32DC009975-11

Keywords: Inner ear, Epigenetics, Comparative genomics

Poster: 714

EFFICIENT SENDAI VIRUS CLEARANCE AFTER REPROGRAMMING

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Non-integrative reprogramming technologies promise a consistent and efficient reprogramming of somatic cells into induced pluripotent stem cells (iPSCs), with applications to disease modeling, drug discovery, and cellular therapy. Sendai Virus has been shown to efficiently reprogram a variety of cell types and generate quality footprint-free iPSCs. However, Sendai virus clearance is a pervasive challenge yet to be adequately addressed on a large scale, requiring several passages to eliminate the virus on bulk cell passages during expansion, where manual cloning is either not practical or timely enough. Foregoing virus clearance jeopardizes the safety of iPSCs in crucial downstream applications including the manufacture of biological products for cellular therapy. In this study, we collected blood from 40 individuals, purified the peripheral blood mononuclear cells, and expanded the erythroblast population for reprogramming with Sendai Virus. Three weeks after reprogramming, iPSCs were passaged manually for clonal expansion based on morphological features. IPSCs colonies were cloned for 3 to 10 passages to determine the earliest passage at which more than 90% of the clones are Sendai-free. The iPSCs were tested for Sendai virus clearance by qRT-PCR. Our results indicate that around 90% of the clones cleared the viral backbone by passage 7 and 100% by passage 10. The iPSCs clones were pluripotent, able to differentiate into the three germ layers, and karyotypically normal. These results indicate manual cloning is an effective method to ensure rapid and effective Sendai clearance. Importantly, our work serves as a reference study on the efficacy-to-resource trade-offs in passage-based Sendai virus clearance within the context of iPSCs reprogramming.

Keywords: Reprogramming, Sendai, iPSCs

Poster: 715

DIFFERENTIAL EPIGENETIC REPROGRAMMING POTENTIAL AMONG PLURIPOTENT, GERM AND SOMATIC CELL TYPES

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In vitro spermatogenesis, derived from either spermatogonial stem cells (SSCs) or induced pluripotent stem cells (iPSCs), represents a potential solution for male infertility caused by defective male gametogenesis. The establishment of an accurate, efficient, and reproducible in vitro cell system that recapitulates normal male germ cell development in vivo has been a goal in the fields of Reproductive Sciences. Although there has been significant progress, in vitro spermatogenesis remains an unfulfilled challenge. Studies have shown that epigenetic aberrations can accrue during the reprogramming processes associated with the derivation and/or induction of differentiation of iPSCs. Abnormalities that develop in germ cell-like cells (GC-LCs) derived from iPSCs may result from retention of epigenetic memory and/or defects in epigenetic reprogramming associated with induced transitions in cell fate required to generate iPSCs or GC-LCs. To test this hypothesis, we have developed an in vitro system in which transitions among pluripotent, germ and somatic cell states can be induced and monitored at the epigenomic level with the goal of optimizing conditions to recapitulate in vitro the epigenetic reprogramming that normally accompanies male germ cell development in vivo. As a first step, we derived iPSC lines from somatic (mouse embryonic fibroblasts [MEFs], adult tail-tip fibroblasts [TTFs]) or germ (primordial germ cells [PGCs] or SSCs) cell types carrying the DOX-inducible 4F2A reprogramming cassette transgene to determine if iPSCs derived from somatic versus germ cell types display differential reprogramming efficiency and/or distinct epigenetic programming. We find that the timing of first iPSC colony appearance differs among iPSC lines derived from different source cell-types as follows (fastest to slowest): PGC > SSC > MEF > TTF, which likely reflects the extent to which epigenetic programming within each of these initial cell types differs from that in the resulting iPSCs. To test this hypothesis, we are conducting comprehensive transcriptomic and epigenomic profiling of iPSC lines derived from each starting cell type. Ultimately, we seek to determine if differences in cell types from which iPSCs are derived will impact the efficiency and/or quality of GC-LCs that can be derived from each in vitro.

Keywords: induced pluripotent stem cells, epigenetic reprogramming, germ cells

Poster: 716

THE DEVELOPING HUMAN ODONTOBLAST: UNRAVELING PROGENITOR SOURCES AND SPATIOTEMPORAL SIGNALING VIA SINGLE CELL RNA SEQUENCING

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Tooth structure lost due to caries, trauma or genetic disorders is currently restored by artificial prosthesis. To bioengineer natural tooth structure, increased knowledge of the signaling pathways responsible for tooth development is required. We generated a novel single cell atlas of the fetal human toothgerm to assess transcriptome heterogeneity and delineate cell lineage relationships in early development, differentiation, and fate determination via single-cell combinatorial indexing RNA sequencing (sci-RNA-seq). The transcriptomic signatures of tooth germs representing early to late tooth development (gestational weeks 9-22) were analyzed via Monocle3. Clustering, top gene expression and pseudotime analysis identify a heterogeneous population of five transcriptionally unique dental mesenchyme cell groups: dental ectomesenchyme (PRRX1, RUNX2, JAG1), preodontoblast (WNT5A, NUPR1, NOTUM), odontoblast (DSPP, SALL1, S100A13), subodontoblast progenitor (SOX5, FGF10), and subodontoblast (FBN2, FGF3, PTCH1). Real-time density and pseudotime analysis confirm that preOB gives rise to OB. SubOB shares a similar gene expression profile to preOB, however subOB arises later in development. Therefore, we propose that OBs are formed in two developmental waves; subOB are a reserve population that can act functionally as preOB to generate lost OB. Immunohistochemical analysis reveals spatiotemporally specific expression of OB marker DSPP and ameloblast markers AMELX, AMBN and SP6. Interestingly, distinct synchrony is observed between marker expression patterns of the developing OB and ameloblast, indicating intercellular signaling during development. Differential gene expression in the OB and subOB lineages highlight the transcriptional regulation of their differentiation and the need to identify the underlying regulatory mechanisms involved, allowing for development of therapeutic practices, a novel HiPSC to OB differentiation protocol, and ultimately a tooth organoid.

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Keywords: Odontoblast, Regenerative dentistry, single cell RNA sequencing

Poster: 717

MOLECULAR DYNAMICS OF HUMAN HIPPOCAMPAL NEURAL STEM CELLS DURING DEVELOPMENT

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The human hippocampus (HP) is known to be key for important cognitive functions such as memory and learning. Yet, the molecular and cellular processes that orchestrate its formation remain unknown. Different populations of neural stem cells (NSCs), the foundation of neurogenesis, drive the development of the HP through staggered migration waves during the gestational and early postnatal period. In rodents and other mammals, neurogenesis is maintained throughout the entire lifetime due to NSCs that remain in the dentate gyrus (DG). However, in the human DG neurogenesis is absent from the early childhood onwards and an adult neurogenic niche is never formed, suggesting the presence of differential dynamics during the formation of the human DG that impede the long-term establishment of the NSCs. In the present study, we implement transcriptomic sequencing combined with histological validation to unravel the molecular signatures of the developmental NSCs in the human HP, for the proper identification of the potential subpopulations. We analyzed 7 different samples covering a wide age range from the mid-gestational period (GW15, GW19, GW21), early postnatal period (2weeks, 4 months and 7 months) and adulthood (38 years). Our preliminary data revealed the presence of distinct neuronal populations (Granular, pyramidal and interneurons) and well-differentiated glial clusters, including astroglia and RGCs. Isolating and re-clustering the nuclei with high gene expression for known NSC markers we identified the presence of heterogeneous populations of NSCs and progenitors during the HP development. Together with our histological analysis, the data supports a model where HP progenitors switch through different cell states during mid-gestation and are rapidly depleted during the first years of life. Our results allow the study of the development of hippocampal NSCs from a comparative perspective, addressing the similarities and pointing out the key differences between the well-studied rodent model and the human. The similar migration patterns of RGCs and NSCs in both models, together with the existence of NSCs during early childhood pose intriguing questions including how the HP neurogenic niche is regulated and avenues that can modulate the process.

Funding Source: NIH P01NS083513 and The Roberta and Oscar Gregory Endowment in Stroke and Brain Research

Keywords: Human, Hippocampus, Neurogenesis

Poster: 718

INCREASED PROLIFERATIVE AND DIFFERENTIATION CAPACITY OF PLACENTA DERIVED MESENCHYMAL STEM CELLS FROM MEDIAN MATERNAL AGES CORRESPOND TO TELOMERE SHORTENING

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Adult-MSCs hold great promise for regenerative medicine. However, with systemic aging it is well known that MSCs experience functional decline. Age-related changes involve loss in proliferative potential, differentiation ability and increased senescence. Furthermore, these properties may also vary depending on the source of MSCs. Placenta-Derived Mesenchymal Stem Cells (PDMSCs) are great source of MSCs. However, the effect of maternal age on PDMSCs is not well known. Therefore, a thorough characterization of phenotypic changes and differentiation potential of in-vitro culture of MSCs isolated from increasing maternal age is required. The aim of the study is to underscore the effect of maternal age on cellular biology characteristics of the morphological, proliferative and differentiation capacity of PDMSCs, particularly isolated from chorion villi of the placenta. Moreover, groups studied involve short age ranges (18-21, 22-25, 26-30,31-35 and 36 and over) to gain a better understanding of which reproductive stage present higher regenerative potential. PDMSCs were isolated, cultured and multipotency markers CD105, CD90 and CD73 were characterized by flow cytometry showing significantly higher expression in cells isolated from maternal age between 22-35. Self-renewal was assessed by cumulative population doubling (CPD), cell growth-curve (CGC) and colony forming assay-fibroblast (CFU-F) resulting in higher proliferation rates in maternal ages 22-35. Pluripotency markers, differentiation and telomere length were evaluated via qRT-PCR. Pluripotency markers showed higher expression in median maternal ages (22-35) consistent with results of multipotency markers. Interestingly, results showed a ~2-3 fold change increase in differentiation assay for chondrocytes and osteocytes lineage in cells isolated from maternal age 31-35, which is important to take in account for orthopedic applications. Moreover, cells isolated from maternal ages between 22-35 presented ~4 fold change in telomere length negatively correlated with results from cell proliferation assays. Where higher proliferative cells presented shorter telomeres. A clear understanding of the effect of maternal age on stem cells regenerative potential will assist on increasing the effectiveness of cell therapies in the future.

Funding Source: Sistema Nacional de Investigación, Convocatoria Continua de Fomento a la Inserción de Talento Especializado from the Secretaría Nacional de Ciencia Tecnología e Innovación de Panamá and Ministerio de Economía y Finanzas.

Keywords: Human Placenta, Lineage differentiation, Telomere Length

Poster: 719

DIFFERENTIATION OF PRIMED HUMAN PLURIPOTENT STEM CELLS EXCLUSIVELY INTO TROPHECTODERM AND GENERATION OF SELF-RENEWING TROPHOBLAST STEM CELLS

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The in vivo embryonic counterpart of cultured human pluripotent stem cells (hPSC) are the cells of the post-implantation epiblast, which give rise to the embryo proper and presumed to no longer be able to develop into trophectoderm (TE), an extraembryonic lineage. Access to well-characterized placental cells for biomedical research is still very limited, largely depending on primary fetal tissues or cancer cell lines. Here, we developed novel strategies under chemically defined conditions that enable highly efficient TE specification from primed hPSC, followed by generation of cytotrophoblast (CTB), multinucleated syncytiotrophoblast (STB), self-renewing trophoblast stem cells (TSCs) capable of differentiating into terminal cell types – syncytiotrophoblast and extravillous trophoblast. Using a broad range of methods, we validated stepwise induction of lineage-specific genes and confirmed characteristic features of placental cells including morphology at the ultrastructural level, bulk and single-cell RNA sequencing and appropriate immunophenotypes. To demonstrate reproducibility, various cell lines were differentiated into TE and then proliferative TSC lines, which maintained their molecular identity after expansion for several months and expressed micro-RNAs of the trophoblast-specific chromosome 19 miRNA cluster. Our data provide strong evidence that primed hPSCs, including induced pluripotent stem cells (iPSCs), can be directly converted into trophoblast, thereby providing an unlimited source of the diverse placental cells suitable for disease modeling, drug screening, and cell-based therapies. These findings also firmly establish that routinely cultured hPSCs have broader developmental competence than previously anticipated.

Keywords: human pluripotent stem cells, trophectoderm, trophoblast stem cells

Poster: 720

STOCHASTIC ACTIVATION OF MMP9 BY THE TRANSCRIPTION FACTORS IRF6 AND OVOL1 IS REQUIRED FOR MESENCHYMAL TO EPITHELIAL TRANSITION DURING CELLULAR REPROGRAMMING

Valakos, Dimitrios, Polydouri, Ioanna, Klagkou, Eleftheria, Vassaki, Katerina, Papathanasiou, Maria, Papadopoulou, Deppie, Sianidis, Georgios, Vatsellas, Giannis, Agelopoulos, Marios, Thanos, Dimitris

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Cellular reprogramming by OSKM overexpression towards induced pluripotent stem cells (iPSCs) requires the construction of a gene regulatory network composed of 9 transcription regulators (9TR GRN). Of these, Irf6 and Ovol1 are directly induced by OSKM and are co-expressed stochastically in early iPSC colonies. Herein, we have generated GFP reporters bearing the enhancers of Irf6 and Ovol1, which faithfully recapitulate the expression pattern of the endogenous genes during cellular reprogramming. We used these reporters as tools and isolated GFP+ and GFP- subpopulations of cells at day 6 of reprogramming for both Irf6 or Ovol1 expressing cells, and demonstrated that the GFP+ cells have a two-fold higher probability to reach the pluripotent state as compared to GFP- cells. Subsequently, RNA-seq analysis of these two cell populations revealed a significant enrichment for genes expressed during the Mesenchymal to Epithelial Transition (MET). Consistent with this is our finding that Irf6 or Ovol1 shRNA knockdown blocks MET transition and therefore reprogramming. Importantly, we found that Irf6 and Ovol1 expressing cells share a common network composed of 23 differentially expressed genes, where the central node is the matrix metallopeptidase-9 (Mmp9)/ (MMP9) protein. ChIP-seq experiments revealed that Mmp9 expression is directly controlled by both Ovol1 and Irf6 transcription factors, a result consistent with their co-existence in early iPSCs colonies. Pharmacological inhibition of MMP9 during fibroblast reprogramming decreased the efficiency of reprogramming significantly, due to induced apoptosis of cells within the early iPSCs colonies. Remarkably, pharmacological inhibition of MMP9 during the reprogramming of hepatocytes had no effect in reprogramming, a result consistent with the fact that these cells are epithelial, and therefore do not require MET to achieve pluripotency. In summary, our data are consistent with a model where the stochastic activation of the MMP9 by the 9TR GRN in early iPSCs colonies is essential for MET. Our model predicts that the enzymatic activity of MMP9 facilitates the detachment of cells undergoing reprogramming from the extracellular matrix to promote cell-cell junctions and acquire the epithelial state, a prerequisite for the completion of cellular reprogramming.

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Keywords: Transcription Factors, Gene Regulatory Networks, Cellular Reprogramming

Poster: 919

DECODING HUMAN MEGAKARYOCYTE DEVELOPMENT

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Despite our growing understanding of embryonic immune development, rare early megakaryocytes (MKs) remain relatively understudied. Here we used single-cell RNA sequencing of human MKs from embryonic yolk sac (YS) and fetal liver (FL) to characterize the transcriptome, cellular heterogeneity, and developmental trajectories of early megakaryopoiesis. In the YS and FL, we found heterogeneous MK subpopulations with distinct developmental routes and patterns of gene expression that could reflect early functional specialization. Intriguingly, we identified a subpopulation of CD42b+CD14+ MKs in vivo that exhibit high expression of genes associated with immune responses and can also be derived from human embryonic stem cells (hESCs) in vitro. Furthermore, we identified THBS1 as an early marker for MK-biased embryonic endothelial cells. Overall, we provide important insights and invaluable resources for dissection of the molecular and cellular programs underlying early human megakaryopoiesis.

Keywords: human megakaryopoiesis, heterogeneity, THBS1

12:00 - 13:00 EDT

POSTER SESSION 7

MODELING DEVELOPMENT AND DISEASE

Poster: 588

THREE CELL-TYPE CARDIAC MICROTISSUES PROMOTE POST-NATAL SCN5A ISOFORM EXPRESSION IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES AND REVEAL THE MUTANT PHENOTYPE

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Immaturity of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) is presently an obstacle in using them to model adult- or post-natal-onset cardiac diseases. In conventional two-dimensional cultures, hiPSC-CMs have fetal-like electrophysiological features and express mainly fetal isoforms of developmentally regulated genes. Mutations in the cardiac sodium channel gene SCN5A cause distinct cardiac arrhythmias and the gene undergoes a fetal-to-adult isoform switch postnatally; it is therefore difficult to understand in vitro how specific mutations affect cardiomyocyte electrophysiology. To examine the electrophysiological consequences of a mutation in the adult isoform of SCN5A by promoting hiPSC-CM maturation to a postnatal state using a new three-dimensional cardiac microtissue model containing hiPSC-derived cardiac fibroblasts and endothelial cells. hiPSC-CMs were derived from a patient carrying two compound mutations in SCN5A: p.W156X in exon 4 and p.R225W in the adult splicing variant of exon 6 (exon 6B). Using CRISPR/Cas9, we corrected the exon 4 mutation to investigate specific effects of p.R225W mutation. ddPCR analysis showed a small fraction of exon 6B-containing transcripts in hiPSC-CMs and no electrical changes as assessed by single-cell patch clamp electrophysiology. Engineering the hiPSC isogenic pair by excising exon 6A with CRISPR/Cas9 also

did not increase adult SCN5A expression but rather resulted in splicing impairment and strongly reduced sodium current. However, maturing hiPSC-CMs in cardiac microtissues promoted SCN5A exon 6B expression and revealed the contribution of both SCN5A mutations to the cellular disease phenotype. Our results confirm that immaturity of conventional hiPSC-CMs precludes measurement of the electrical phenotype caused by mutations in the adult isoform of SCN5A but that this can be overcome by promoting maturation using cardiac microtissues. This facile refinement to conventional hiPSC-CM models opens new possibilities to study developmentally regulated cardiac genes and postnatal phenotypes; specifically, the underlying mechanism of cardiac arrhythmia in genetic diseases of adults.

Funding Source: Netherlands Organisation Health Research & Development ZonMW (MKMD no. 114022504); Transnational Research Project Cardiovascular Diseases (JTC2016_FP-40-021 ACM- HF); Health~Holland TKI-LSH PPP-allowance (LSHM17013-H007).

Keywords: Cardiac arrhythmia, engineered cardiac microtissues, Brugada Syndrome

Poster: 730

MOUSE SKELETAL STEM CELL FATE DEFECTS CAUSED BY PDGFRB ACTIVATING MUTATION WITH OSTEOPENIA AND OVERGROWTH

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Autosomal dominant PDGFR gain-of-function mutations in mice and humans cause a spectrum of wasting and overgrowth disorders afflicting the skeleton and other connective tissues, but the cellular origin of these disorders remains unknown. We demonstrate that skeletal stem cells (SSCs) isolated from mice with a gain-of-function D849V point mutation in PDGFR exhibit SSC colony formation defects that parallel the wasting or overgrowth phenotypes of the mice. Single-cell RNA transcriptomics with the SSC colonies demonstrates alterations in osteoblast and chondrocyte precursors caused by PDGFR D849V. Mutant SSC colonies undergo poor osteogenesis in vitro and mice with PDGFR D849V exhibit osteopenia. Increased expression of Sox9 and other chondrogenic markers occurs in SSC colonies from mice with PDGFR D849V. Increased STAT5 phosphorylation and overexpression of Igf1 and Socs2 in PDGFR D849V SSCs suggests that overgrowth in mice involves PDGFR D849V activating the STAT5-IGF1 axis locally in the skeleton. Our study establishes that PDGFR D849V causes osteopenic and overgrowth skeletal phenotypes that are associated with intrinsic changes in SSCs.

Funding Source: Hae Ryong Kwon was supported by F32-HL142222 from NIH/NHLBI. This work was supported by US NIH grant R01-AR073828 (Lorin E. Olson) and grants from the Oklahoma Center for Adult Stem Cell Research – a program of TSET (Lorin E. Olson).

Keywords: PDGFR beta function on skeletal stem cells, Stem cell study with single-cell RNA seq, Penttinen and Kosaki overgrowth syndrome

Poster: 731

CARDIAC DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A DESMINOPATHY PATIENT FOR IN VITRO DISEASE MODELING

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Desmin (DES) is a type III intermediate filament of adult striated muscle that regulates gene expression, organelles performance and fiber contraction. Desmin-related diseases are termed desminopathies and involve cytoplasmic protein aggregates that generate cardiomyopathy. We propose to model this disease in vitro by differentiating to cardiomyocytes a pluripotent stem cell (iPSC) line derived from a patient with a desminopathy (DES-J). DES-J has heterozygous triplet duplication in DES exon 6 (Glu353 incorporation) not previously described that impairs normal desmin structure. DES-J iPSC line was characterized with a normal karyotype, expression of pluripotency genes and ability to differentiate to the three germline layers in embryoid bodies analyzed by RT-qPCR and immunofluorescence. DES-J iPSC line was then differentiated to cardiomyocytes using a monolayer protocol. In order to associate the mutation to disease, we analyzed DES alleles expression during cardiac differentiation and we determined that both normal and mutated alleles are expressed in iPSCs and after cardiac mesoderm induction. Afterwards, we confirmed by RT-qPCR that pluripotency genes (NANOG, OCT4) are downregulated whereas mesendoderm (TBXT), mesoderm (NKX2.5, MESP1) and cardiac genes (TNNT, VIM, CX43) are upregulated at days 3.5 and 14 of cardiac protocol. Flow cytometry analysis revealed that 75% of the cells were CD56+ (mesoderm) by day 3.5 and 35% were TNNT+ (cardiomyocytes) by day 14 of differentiation, similar to control iPSCs. Moreover, we analyzed gene expression of desmosomal, mitochondrial, intermediate filaments, ubiquitin and other desmin interacting proteins throughout differentiation protocol of DES-J and control cells. Interestingly, we observed different gene expression profiles between both cell lines including lower expression of desmosomal and intermediate filaments genes in DES-J respect to control from day 0 to day 23 of differentiation. In conclusion, we generated an iPSC line from a desminopathy patient with a novel mutation, which was able to differentiate to cardiomyocytes. Despite preliminary results showed differences in the cardiac differentiation process respect to normal cells, we could obtain cardiomyocytes which will be studied in order to model in vitro the disease.

Keywords: iPS CELL LINE OF DESMINOPATHY PATIENT, CARDIAC DIFFERENTIATION, IN VITRO DISEASE MODELING

Poster: 732

EXTRACELLULAR MATRIX MEDIATED MATURATION OF CHAMBER SPECIFIC HIPSC-CM MONOLAYERS

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To date in vitro cardiotoxicity screening using hiPSC derived cardiomyocytes has been validated using monolayer mixtures of atrial, ventricular, and nodal cells with predominantly immature fetal like phenotypes. This is not an optimal representation of the human heart, where chamber specific cells perform critically specialized functions. Our objective here was to generate purified monolayers of mature atrial or ventricular hiPSC-CMs for electrophysiological study using voltage sensitive dyes. To this end, we differentiated hiPSCs (19-9-11) to cardiomyocytes (CMs) using a published small molecule approach for cardiac chamber specific cell specification. CM purification was done using magnetic bead activated cell sorting and cells were replated as confluent monolayers for electrophysiology functional assessment. CM phenotype was matured using perinatal stem cell derived extracellular matrix coated plates (MatrixPlus), while fetal phenotype was maintained on a mouse cell derived extracellular matrix (Matrigel) for comparison. Action potential duration (APD90) was shorter in atrial cardiomyocytes (Matrigel=456.3±75.9ms, n=6 and MatrixPlus=474.6±46.8ms, n=6) than in time matched ventricular cardiomyocytes (Matrigel=551.5±114.7ms, n=6 and MatrixPlus=548.3±12.7ms, n=6). Although maturation state did not impact APD90, APD30 was significantly prolonged in both atrial and ventricular hiPSC-CM monolayers matured on Matrix Plus (e.g., Atrial Matrigel=173.2±11.4ms, n=6 and Atrial Matrix Plus=308.7±20.1ms, n=6; t test P<0.0001.). The shift of APD30 resulted in lower AP triangulation values for atrial (Matrigel=0.62±0.08, n=6 and Matrix Plus=0.35±0.03, n=6) and ventricular CMs (Matrigel=0.65±0.05, n=6 and MatrixPlus=0.43±0.08, n=6) matured on Matrix Plus ECM. All groups responded to hERG channel block (100nM E-4031) with APD prolongation, only the ventricular CMs matured on MatrixPlus responded with Torsades de Pointes (TdP)-like activation patterns. Expansion of this process to high throughput screening plates (96wp) will make it possible to develop atrial specific drugs and ventricular specific drugs using a more optimal in vitro system that more closely mimics the in vivo conditions of the working heart.

Funding Source: NIH SBIR from NIEHS (R44ES027703/NH/NIH HHS/United States)

Keywords: Cardiotoxicity, Cardiomyocytes, Electrophysiology

Poster: 733

PCP4 IS EXPRESSED IN A SUBPOPULATION OF CARDIAC CONDUCTION SYSTEM CELLS IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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The Cardiac Conduction System (CCS) is a heterogenous network of specialized cells that control cardiac rhythm through initiating and synchronizing the excitation and contraction of atria and ventricles. Previous studies have demonstrated that cells of the ventricular CCS are the main inductors of life-threatening heritable and acquired arrhythmias. However, the mechanisms underlying these proarrhythmic events still remain poorly understood due to the challenge of isolating and identifying this rare population of cells. The ventricular CCS is formed by Purkinje cells (PC) and comprises about one percent of all adult ventricular cardiomyocytes. PCP4, Purkinje cell protein 4, has recently been described as a marker for the mature cardiac Purkinje fiber network in the mouse heart. Here, a dual fluorescent protein-reporter system was generated using human induced pluripotent stem cells (hiPSC) to identify CCS-like cells. Using CRISPR-Cas9, Red Fluorescent Protein (RFP) was targeted into the PCP4 locus and enhanced Green Fluorescent Protein (EGFP) was expressed under control of the pan-cardiomyocyte marker TNNT2. Directed cardiac differentiation resulted in a sub-population of about one percent double-positive cells (1.04% ± 0.74 at day 60, n=12) that displayed immature features of cardiac PC. Bulk and Single cell RNA-Seq were used to characterize the transcriptomic profile of this sub-population. Established PC markers, including PCP4, GJA5 and SCN10A, displayed increased expression in the double positive population. Furthermore, upregulation of previously described mouse ventricular CCS PC transitional markers, including SLIT2 and SNAI2, was observed. Electrophysiological studies revealed immature human action potential (AP) properties for RFP/GFP+ cells (90% AP repolarization (APD90): 235.9±71.5ms, AP amplitude (APA): 95.6mV±7.5, resting membrane potential (RP) -55.9±5.0ms ; n=5). In summary, the dual-reporter model developed here provides a new platform to isolate rare CCS-like cells, perform high-throughput downstream translational studies, and optimize the generation of PC from hiPCS. This platform is the first human PC reporter system and offers the unique possibility to improve insight into human CCS development and pathophysiology.

Funding Source: NYS/NYSTEM, IDEA C32603GG NIH/NCI, P30CA016087

Keywords: cardiac conduction system, hiPS-derived cardiomyocytes, cardiac development

Poster: 734

GENERATION OF FUNCTIONAL ENDOCARDIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING BMP10 SIGNALING

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Endocardial cells represent a specialized population of endothelial cells that line the chambers of the heart. During embryonic cardiac development, these cells play a key role as they are responsible for inducing the formation of the first functional population of cardiomyocytes, known as trabecular cardiomyocytes. In the absence of endocardium, the trabecular myocardium fails to develop, resulting in severe malformations in the heart and embryonic lethality. In addition to this inductive role, the endocardial lineage is the source of several other cell types in the heart including the cells that form the valves and the endothelial cells that make up a portion of the coronary vasculature. Given this potential, the ability to generate human endocardial cells could provide unique opportunities for disease modeling and cell therapy. To access human endocardial cells, we developed a protocol that promotes the generation of NKX2-5+CD31+ endothelial cells from human pluripotent stem cell (hPSC)-derived cardiovascular progenitors. These cells express the cohort of genes that identifies the endocardial lineage in vivo — NKX2-5, GATA4, GATA5, NFATC1, NPR3 and NRG1 — 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, as well as the capacity to undergo EndoMT to give rise to mesenchymal cells that express markers of the valvular interstitial cells (VIC). Our analyses of the signaling pathways required for the development of hPSC-derived endocardial cells identified a novel role for BMP10 as a key regulator of the specification of the endocardial lineage from cardiovascular mesoderm. In summary, the findings presented in this report describe a method for the derivation of endocardial cells from hPSCs and in doing so, add to the growing list of cardiovascular cell types that can be generated using this in vitro platform.

Funding Source: This work was supported by grants from the Canadian Institute of Health Research (PJT148717, PJT156432, and FDN159937).

Keywords: Endocardium, Heart valves, Cardiomyocytes

Poster: 735

CARDIAC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS USING DEFINED EXTRACELLULAR MATRIX PROTEINS REVEALS ESSENTIAL ROLE OF FIBRONECTIN

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Prior studies have defined the role of soluble factors in the differentiation of human pluripotent stem cell to cardiomyocytes (hPSC-CMs), but the role of extracellular matrix (ECM) proteins has not been systematically investigated. We previously described a matrix sandwich method in which hPSCs were subjected to an overlay of Matrigel™ which improved cardiac differentiation by enhancing the formation of Brachyury+ mesoderm. Matrigel™ is an undefined mixture of ECM proteins and exhibits lot-to-lot variability. The present study tests the ability of defined ECM proteins to support cardiac differentiation of hPSCs. We found that fibronectin (FN), laminin 111 (LN111), and laminin 521 (LN521) all enable the attachment and expansion of hPSCs. Growth factor (Activin A, BMP4, and bFGF) stimulated cardiac differentiation on the defined ECM was specifically enhanced by FN overlay; however, overlays of LN111 and LN521 inhibited cardiac differentiation. Efficient cardiac differentiation of hPSCs grown on FN and LN111 occurred without a matrix overlay. Evaluation of endogenous ECM production determined that hPSCs produced significant levels of endogenous FN. Doxycycline-induced shRNA knockdown of FN demonstrated that FN was required at the initial stage of differentiation to form Brachyury+ mesoderm, and exogenous FN rescued the genesis of Brachyury+ cells and hPSC-CM differentiation. Antibodies blocking FN binding to integrin β1, but not α5, significantly inhibited the cardiac differentiation in a concentration-dependent manner. Furthermore, inhibition of integrin-linked kinase (ILK) by small molecule Cpd22 also significantly inhibited the cardiac differentiation in both the growth factor and small molecule based cardiac differentiation protocols. These results demonstrate that FN, LN111, and LN521 enable the seeding and cardiac differentiation of hPSCs and that FN is essential in early stages of cardiac differentiation of hPSCs acting via integrin β1 and ILK signaling.

Funding Source: NIH R01 HL129798 (T.J.K.) and NIH U01HL134764 (T.J.K.)

Keywords: Human pluripotent stem cells, Fibronectin, Precardiac mesoderm

Poster: 736

ASSESSING SIGNALING OUTCOMES AT THE SINGLE-CELL LEVEL IN PRIMARY RAT NEONATAL AND HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Diseases affecting the cardiovascular system are prominent representing leading causes of morbidity and mortality in the western world. The heart is arguably the most vital organ in the human body consisting of more than 10 cell types, however, diseased tissue/cells are relatively inaccessible making human in vitro studies challenging. Access to healthy- and patient-derived induced pluripotent stem cells (iPSCs) thus represents a unique model system to study the underlying molecular drivers of disease. Considering that iPSC-cardiomyocytes (iPSC-CMs) represent a relatively "new" model, we are interested in comparing them to an established primary cell model, neonatal rat ventricular cardiomyocytes (NRVMs). Using FRET-based biosensors, we have been able to assess kinase activity (ERK, PKA) in NRVMs and iPSC-CMs from control donors using a panel of drugs that target α-adrenergic, β-adrenergic and angiotensin receptor systems, representing drugs prescribed to heart failure patients. At the single cell level, differences in ERK1/2 signaling have been observed between cell types but also when measuring FRET responses in iPSC-CMs cultured for different lengths of time. The level of maturity of these cells is thus important to bear in mind when modeling cardiovascular diseases of adult-onset. For instance, dilated cardiomyopathy, characterized by systolic dysfunction and ventricle dilation is a leading cause of heart failure, yet its etiology is often idiopathic with no clear genetic insult. Several lines of evidence have already reported reduced PKA activity in iPSC-CMs derived from patients suffering from DCM. Here, our aim is to build a comprehensive map of signalling outcomes (ERK, PKA, Ca2+, PKC, etc...) combined with phenotypic assays in patient versus control iPSC-CMs, while taking cardiomyocyte maturity into consideration. We anticipate that the pathophysiological mechanism leading to the diseased state may not be unique across different patient samples affected by idiopathic DCM but patients may be grouped into patterns of signaling alterations. Identifying dysregulated pathways may help inform drug discovery and allow us to develop rational and biology-based therapeutic strategies approaching personalized medicine.

Funding Source: This project is supported by the Canadian Institutes of Health Research (CIHR) and the Courtois Foundation.

Keywords: Cellular signaling, Single-cell FRET, Dilated cardiomyopathy

Poster: 737

MECHANISMS OF CADMIUM-INDUCED ABERRANT DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO CARDIOMYOCYTES AND CARDIAC ORGANOID FORMATION MIMICKING HEART DEVELOPMENT

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Cadmium (Cd) is a widespread environmental contaminant. Cd exposure is associated with cardiovascular diseases, and maternal exposure to Cd is a significant risk factor for congenital heart disease (CHD). However, the mechanisms of Cd on developmental cardiotoxicity are not well-defined. Embryonic stem cells (ESCs) offer an excellent opportunity for studying the mechanisms of developmental toxicity. 3D aggregates of ESCs, called embryoid bodies (EBs), can recapitulate events involved in early embryogenesis such as germ layer formation. Here, we found that a 7-day exposure to a human-relevant, non-cytotoxic dose (0.6 mM; 100 ppb) of Cd inhibited differentiation of EBs to ectoderm and mesoderm germ layers via suppression of several genes (WNT1, WNT3, GSK3B, CTNNB1) associated with the Wnt/beta-catenin signaling pathway. NKX2-5 is a transcription factor that plays key roles throughout heart development and formation, and mutations can lead to atrial and ventricular defects. To explore potential adverse effects of Cd on cardiomyocyte formation we used a 2D ESC line containing an NKX2-5 reporter to model mesoderm differentiation and cardiac induction. Using an 8-day differentiation protocol, Cd (0.15 μM; 27 ppb) suppressed cardiac induction and cardiomyocyte differentiation. Compared with controls, Cd down-regulated mesoderm-associated transcription factors (MESP1, MIXL1, EOMES), mesoderm markers (HAND1, SNAI2, HOPX, and FOXA2), and cardiac-specific genes (NKX2-5, GATA4, Troponin T, and ±-Actinin). To mimic the structure and function of the heart during development, the NKX2-5 reporter ESCs were used to form cardiac organoids composed of cardiomyocytes (TNNT2+), cardiac fibroblasts (THY1+), endothelial (PECAM1+) and endocardial cells (NFATC1+). Cd dramatically decreased cardiac organoid contraction and inhibited expression of genes associated with functional cardiomyocytes such as conduction (HCN4), contractile function (MYH7) and calcium handling (CACNA1C). In conclusion, low-dose Cd suppressed mesoderm formation through Wnt/^{2}-catenin signaling pathways and thus inhibits downstream cardiomyocyte differentiation and cardiac induction. These studies provide valuable insights into the cellular events and molecular mechanisms associated with Cd induced CHD.

Keywords: Embryonic stem cell, cardiomyocytes, cardiac organoid

Poster: 738

MECHANISMS OF INHERENTLY LOW FIDELITY OF CHROMOSOME SEGREGATION IN HUMAN PLURIPOTENT STEM CELLS

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Fidelity of chromosome segregation is crucial in cell division and chromosomes must be accurately segregated to produce euploid daughter cells with an equal distribution of chromosomes. Errors in chromosome segregation result in the gain or loss of whole chromosomes, producing aneuploid cells with abnormal numbers of chromosomes. In normal human somatic cells, chromosome segregation is regulated so that aneuploidy is rare. In contrast, during human development, chromosome segregation errors are surprisingly common in human pluripotent embryonic cells, resulting in aneuploidy being the leading cause of miscarriages and birth defects. Yet, the underlying mechanisms remain poorly understood, especially for mitotic errors. Here, we directly compare mitosis in human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), and somatic cells to investigate the causes of chromosome segregation errors in hPSCs. Using quantitative live-cell imaging, immunofluorescence microscopy and chemical approaches, we show that mitotic error rates are significantly elevated in hPSCs compared to somatic counterparts, with lagging chromosomes being the most common error. We further demonstrate that improper chromosome microtubule attachments cause lagging chromosomes in hPSCs. In addition, we use chemical compounds to show that decreasing chromosome microtubule attachment stability or prolonging mitotic duration decrease the frequency

of mitotic errors in hPSCs. Thus, our results demonstrate that chromosome microtubule attachment error correction is inefficient in hPSCs accounting for the high mitotic error rates, but we can improve error correction efficiency by decreasing chromosome microtubule attachment stability or by prolonging mitosis. Importantly, our results provide new strategies for how to improve the genome stability of hPSCs growing in culture which is critical for the success of regenerative and reproductive medicine.

Funding Source: Hitchcock Foundation Pilot grant and NIH R01HD101436 to KG

Keywords: Aneuploidy, Mitosis, Human Pluripotent Stem Cells

Poster: 739

CONNEXINS IN HUMAN STEM CELL LINEAGE SPECIFICATION

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During embryonic germ lineage commitment, cells must communicate with each other to ensure proper tissue development and function. Connexin (Cx) proteins enable direct cell-cell communication through gap junction channels between neighbouring cells. Despite efforts to characterize gap junction expression in stem cells and throughout terminal differentiation, there is little information regarding the expression of connexins during human PSC specification to the three embryonic germ lineages. We screened the expression of 12 human connexin isoforms in undifferentiated human iPSCs and directed differentiation into the three embryonic germ lineages: ectoderm, endoderm and mesoderm. Cx43 is the most highly expressed connexin in hiPSCs as well as cells of each germ lineage. However, Cx43 CRISPR-Cas9 knockout hiPSCs formed all three germ layers at comparable levels to wild-type hiPSCs, suggesting that Cx43 is dispensable during hiPSC lineage specification. Meanwhile, qPCR analysis of WT hiPSCs revealed upregulation of Cx62 in hiPSC-derived ectoderm cells, Cx45 upregulation in mesoderm cells and enrichment of Cx32 and Cx40 in endoderm. Ongoing studies will evaluate whether any connexin species are upregulated in compensation for the loss of Cx43 in our Cx43 KO hiPSCs. This is the first comprehensive analysis of Cx isoform expression throughout human iPSC germ layer differentiation. In summary, several connexins appear to work together to maintain human PSC pluripotency and survival and this cell-cell communication may become more important as pluripotent stem cells undergo cell fate specification.

Funding Source: Faculty of Medicine Memorial University, Natural Sciences and Engineering Research Council

Keywords: Lineage Specification, Connexin, hiPSCs

Poster: 740

LOSS OF TBX3 ENHANCES PANCREATIC PROGENITOR GENERATION FROM HUMAN PLURIPOTENT STEM CELLS

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Tbx3 has been identified as a regulator of fate decisions during liver development in the mouse, but whether it function similarly in humans remains unclear. We generated TBX3 knockout human pluripotent stem cell (PSCs) lines using CRISPR/Cas9 genome editing technology. We differentiated TBX3 knockout lines to hepatocytes and found a decrease in hepatic markers and in hepatocyte function, demonstrating that TBX3 is also important for liver differentiation. Surprisingly, we detected expression of pancreatic markers, including PDX1 in our TBX3 knockout hepatocytes, suggesting TBX3 may regulate liver development by suppressing a pancreatic fate. We next differentiated the TBX3 knockout lines to pancreatic progenitors to determine whether the loss of TBX3 impacts pancreatic differentiation. We found that TBX3 knockout PSCs generated more pancreatic progenitors, and that these progenitors had an enhanced pancreatic gene expression signature at the expense of hepatic gene expression. We also found that epithelial-to-mesenchymal transition was commonly dysregulated in TBX3 mutant pancreas and hepatocyte cells. This suggests that TBX3 may function during EMT in both liver and pancreas development. These data highlight a potential role of TBX3 in distinguishing between hepatic and pancreatic domains during foregut patterning, with implications for enhancing the generation of pancreatic progenitors from PSCs.

Keywords: Pancreas development, Liver development, Endoderm patterning

Poster: 741

A REPRODUCIBLE PROTOCOL FOR THE GENERATION OF BRANCHING LUNG ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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The ability to recapitulate lung development in vitro from human pluripotent stem cells (hPSCs) enables the study of human lung development, as well as the advancement of innovative strategies in respiratory disease modeling, drug discovery, and regenerative cell-based or gene therapies. Current protocols for the derivation of lung organoids from hPSCs are highly variable and lack standardization. To address this, we developed STEMdiffTM Branching Lung Organoid Kit, which provides a streamlined protocol for the reproducible generation of lung organoids across multiple human embryonic (H1, H9, and H7) and induced pluripotent stem cell lines previously maintained in mTeSRTM1 or mTeSRTM Plus. One day after clumps of cells were seeded onto Corning® Matrigel®-coated 24-well plates, the cells were sequentially differentiated over 21 days into the stages of definitive endoderm, anterior foregut endoderm (AFE) buds, and finally lung bud organoids. The lung bud organoids were then harvested and embedded in Matrigel® sandwich layers on Transwell® inserts placed in 24-well plates, with medium feeds added from both the apical and basal sides. The lung bud organoids were further developed into branching lung organoids for 4 weeks. The resulting branching lung organoids developed proximal and distal-like branching airway epithelial structures that expressed EpCAM, CPM, NKX2.1, SOX2, SOX9, MUC1, ACE2, and TMPRSS2 (n=2) as confirmed by immunohistochemistry or flow cytometry. When cultured

for extended periods (> 2 months), there was an increase in expression of distal markers SFTPC, SFTPB, and ABCA3 by RT-qPCR. In addition, the AFE buds cryopreserved on day 7 of the protocol above could readily be differentiated and matured into branching lung organoids. This demonstrated that AFE buds cryopreservation at this stage of the protocol is possible, thus providing a complete workflow. These results demonstrate that STEMdiffTM Branching Lung Organoid Kit is an optimized kit for the recapitulation of lung development that is reproducible across multiple hPSC lines.

Keywords: Organoids, Human Lung, hPSC

Poster: 743

MODELING HYPERBILIRUBINEMIA USING AN ORGANOID SYSTEM

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Neonatal hyperbilirubinemia (NH) is the yellowish discoloration of the skin and sclera due to high bilirubin levels, and it accounts for 114,000 annual deaths worldwide. Nearly 50% of term and 80% of pre-term babies are afflicted with jaundice. The only treatment is 12 hours of phototherapy or exchange transfusion, but they cause other complications. Although several animal models of NH are available, they lack key human proteins (OATP family) and epigenetic control involved in bilirubin metabolism. Moreover, the regulatory mechanism behind UGT1A1 expression, the key rate limiting enzyme of bilirubin metabolism, still eludes us. Here, we use induced pluripotent stem cells (iPSCs) derived human liver organoids (HLOs) to model the disease by treating them with physiologically relevant concentrations of bilirubin (1-10 µg/mL). Healthy HLOs take up unconjugated bilirubin (UCB) efficiently and produce conjugated bilirubin (CB) (UCB: 4.02 ± 0.89 µg/mL P<0.01, CB: 1.86 ± 0.14 µg/mL P<0.01). Our HLOs also express OATP1B1, the human specific transport protein involved in the uptake of UCB. HLO treatment with Cyclosporin A, an OATP1B1 inhibitor, significantly impaired UCB uptake (UCB: 48 % increase, P<0.01), indicating that the bilirubin uptake is not simply by passive diffusion. Since the conjugation of bilirubin was not as effective, we decided to look into signaling pathways involved in UGT1A1 expression in bilirubin treated and control samples using a transcriptomics approach. The RNAseq revealed that several signaling pathways such as Wnt, and PPAR signaling were altered, and could potentially contribute to the regulation of UGT1A1 and low CB. Finally, we treated Crigler-Najjar syndrome (CNS) patient-derived HLOs to model unconjugated hyperbilirubinemia. We found that CNS HLOs were unable to convert it to the conjugated form (UCB: 6.18 ± 1.12 µg/mL P<0.01, CB: 0.48 ± 0.11 µg/mL P<0.01), and therefore grew necrotic over time. In order to remedy this condition, we transfected CNS HLOs with UGT1A1 mRNA. The UGT1A1 transfected CNS HLOs had greater efficiency in clearing bilirubin (UCB: 3.92 ± 0.64 µg/mL P<0.01, CB: 2.17 ± 0.35 µg/mL P<0.01). In conclusion, we demonstrate that our organoid system serves as a tractable, scalable, and easily-manipulatable model for the study and potential therapeutic developments for NH.

Funding Source: NIH DP2-DK128799

Keywords: Human Liver Orgnoids (HLOs), Neonatal Hyperbilirubinemia, UGT1A1

Poster: 744

CELLULAR DECONVOLUTION OF BULK RNA-SEQ TO ESTIMATE CELLULAR COMPOSITION OF IPSC-DERIVED PANCREATIC PRECURSOR CELLS

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Bulk RNA-seq experiments have provided valuable insights into complex biological systems. However, they are typically performed on heterogeneous samples and thus, are confounded by multiple cell types and their different proportions within the sample. To account for this heterogeneity, deconvolution methods have been developed to use single cell RNA-seq (scRNA-seq) gene expression to estimate the cell type proportions of bulk RNA-seq samples. Accounting for this heterogeneity can help clarify downstream analyses and enable a better understanding of cellular functions. In this study, we profiled single-cell and bulk transcriptomes of iPSC-derived pancreatic progenitor cells (iPSC-PPC) and found that distinct clusters of cell types were present, including PPC, replicating cells, early endocrine and exocrine cells. To test if the bulk samples can capture the cellular heterogeneity observed in scRNA-seq, we determined the most variable genes across the cell types and performed cellular deconvolution using MUlti-Subject SIngle Cell deconvolution (MuSiC), where cross-subject and cross-cell consistency were optimized to estimate cell type proportions. We observed high correlations between the cell type proportions calculated on bulk and scRNA-seq, indicating that MuSiC deconvolution reliably captures the cell type differences between samples and that bulk RNA-seq is sufficient to study cell type associations in future large-scale analyses. We then performed leave- one-out cross validation, where a single sample was excluded from the reference at each split, and found that the estimated cell type proportions in the bulk were highly correlated with scRNA-seq. Taken together, this study shows that, although cellular heterogeneity may confound large-scale studies if it is not measured and adjusted for, deconvolution methods can accurately estimate cell type proportions on bulk RNA-seq samples, thereby providing a reliable measure of sample heterogeneity. Furthermore, cellular deconvolution is scalable to hundreds of samples and may be used to study the cell type-specific associations between genetic variation, gene expression and human diseases.

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Keywords: single-cell, pancreas progenitor, deconvolution

Poster: 745

THE EFFECTS OF SURFACTANT AND HYPOXIA ON HUMAN VASCULARIZED LUNG DEVELOPMENT

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Preterm babies born at less than 36 weeks gestation are at risk of respiratory distress syndrome (RDS) due to a deficit of surfactant and lung growth arrest at birth. The fetal lung develops in a hypoxic environment in utero and early exposure to hyperoxia places preterm babies at risk for chronic lung disease. Vascularization is necessary for the normal development and maturation of organs. It is known that paracrine signals from endothelial cells drive lung development and maturation, such as VEGF. In order to better study human lung development in vitro, it is imperative that we mimic the in utero environment. Lung organoids derived from induced pluripotent stem cells (iPSCs) have been useful in modeling lung development and disease, and may provide insight on the effects of hyperoxia on a surfactant deficient vascularized lung organoid (VLO). We have created a human lung platform system using surfactant protein B deficient iPSC lung organoids and co-cultured them with fetal endothelial cells to study lung development in the setting of surfactant deficiency. To elucidate the role of hypoxia on VLOs we have used the RUES2 ESC reporter line (SCGB3A2- mCherry and SPC-GFP) which underwent step wise differentiation into 3D whole lung organoids (WLO). These were then co-cultured with human umbilical venous endothelial cells (HUVECs) for 10 days in both hypoxic (5%) or normoxic (21%) conditions. Flow cytometry for the fluorescent reporters showed an increase in the expression of SPC-GFP but no change in SCGB3A2-mCherry. We also utilized our SFTPB deficient iPSC and CRISPR corrected lines to determine the effects of vascularized lung development in the setting of surfactant deficiency in hypoxia and normoxia. After undergoing stepwise differentiation for 3 weeks, the SFTPB deficient VLOs displayed lower transepithelial resistance than the CRISPR corrected VLOs when analyzed with transepithelial electrical resistance. Further analysis on cellular tight junctions is ongoing. This VLO model system represents the in utero environment more closely and is an unprecedented resource to determine the effects of preterm birth on lung development and disease and may provide us with novel insights into possible therapies to improve long term outcomes.

Funding Source: CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375

Keywords: lung, organoid, hypoxia

Poster: 746

HUMAN LUNG EPITHELIUM DEVELOPMENT AND REGULATION OF AIRWAY HOMEOSTASIS DEPENDENT ON BPIFA1 UPON INCREASED OXYGEN TENSION AND RSV INFECTION

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The human airway epithelium serves as a barrier against foreign pathogens and particulates. At birth, the airway epithelium undergoes rapid physical changes shifting from hypoxic to normoxic air exposure. Premature infants have underdeveloped lung epithelium and are less capable of maintaining epithelial homeostasis and thus have increased likelihood of RSV infections. BPIFA1 has been shown to prevent pathogen induced airway disease severity in mouse models and it would appear to also positively regulate the airway epithelium surface layer. However, the mechanism that drives the expression of BPIFA1 in the developing human airway has not been elucidated. Here we investigate how premature airway development stunts BPIFA1 expression, and how this expression changes upon exposure to increased oxygen tension under both normal and pathophysiologic conditions during RSV infection. First we confirm that BPIFA1 is expressed at the protein level in mature human stem cell derived lung epithelium cultures. Next, we utilize stem cell derived lung models to study airway epithelium development and respective BPIFA1 expression in the later stages of respiratory organogenesis. We then show how BPIFA1 expression is dependent on the change of oxygen tension. Lastly, we infect mature air-liquid interface(ALI) lung cultures with RSV and observe the subsequent change in BPIFA1 protein expression. Future experiments intend on investigating RSV disease severity upon BPIFA1 siRNA knockout. Upon completion of these studies, we will further understand the mechanism that drives BPIFA1 expression during respiratory development and explore the therapeutic potential of targeting BPIFA1 in the premature infant population.

Keywords: Lung Development, Lung Directed Differentiations, Lung Organoids and ALI

Poster: 747

DISEASE MODELING METHODS WITH CANCER PATIENT DERIVED ORGANOIDS

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The use of patient-derived tumor tissue has transformed the field of drug and target discovery research, providing a translational tool and physiologically relevant system to evaluate tumor biology. There has been a transition from 2D assays to 3D cell model systems because they more accurately recapitulate the human in vivo environment and are a more predictive tool for drug discovery. An example of this is the use of patient-derived

organoids (PDO) for oncology research. PDOs are generated from tumor biopsies or surgical procedures and can serve as models to understand patient-specific drug responses and investigate cancer cell growth. Digested tumor pieces and cells harvested from patient-derived tumors exhibit cancer stem cell (CSC)-like qualities and can be expanded over multiple passages to produce large numbers of organoids that maintain molecular characteristics of the original tumor. We present results for breast cancer disease modeling using PDOs formed from primary cells isolated from a patient-derived tumor, TU-BcX-4IC. TU-BcX-4IC represents a rare breast cancer subtype, metaplastic breast cancer (MBC), and is classified as a triple negative breast cancer (TNBC) histological subtype. The tumor exhibited rapid pre-operative growth despite combination neoadjuvant therapy with adriamycin, cyclophosphamide and paclitaxel. PDOs were incubated in the presence of targeted anti-cancer compounds for 24 – 48 hours and then stained for either viability markers or E-cadherin/CD44 markers. Resistance of PDOs to chemotherapeutic agents observed in our experiments were consistent with tumor response in the patient. To facilitate this work we used a Pu·MA System and flowchips for semi-automated organoid assays coupled with high content imaging. The flowchip is equipped with organoid sample wells connected to multiple reservoirs containing assay reagents and allows assays to be performed without disruption to, or loss of 3D sample. This novel assay method using microfluidics enables automation of 3D cell-based cultures that mimic in vivo conditions and allows a wide range of assay detection modalities. The work described here demonstrates the utility of the Pu·MA system for in vitro drug discovery research in testing structurally delicate 3D systems and can be applied to all solid tumor types.

Keywords: Patient Derived Organoid, Cancer Stem Cell, Disease Modeling

Poster: 748

ABNORMALITIES IN THE EXTRACELLULAR MATRIX OF THE HUMAN RETINAL PIGMENT EPITHELIUM IN AN IN VITRO MODEL OF AGE-RELATED MACULUAR DEGENERATION

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Age-related macular degeneration (AMD) is amongst the most common causes of blindness in our aging population, with nearly 200 million cases worldwide today. AMD is in large part triggered by damage to the retinal pigment epithelium (RPE), a cell type that plays a number of critical roles in the support of photoreceptors. The underlying causes of this disorder are not well understood, but there is strong evidence that genetic makeup plays a major role in determining susceptibility to AMD. To understand the early pathogenesis of AMD, we have studied a rare association variant of TIMP3 identified in an AMD genome wide association study (S38C), and independently shown to be causative of a late onset form of Sorsby's Fundus Dystrophy, a related monogenic disorder. We introduced this mutation into human pluripotent stem cells using CRISPR/Cas9 gene editing and differentiated them to RPE cells. Wild type and mutant cells both gave rise to differentiated polarized RPE when grown on transwell filters. We observed a striking phenotype in mutant cells: following several passages in vitro, fully differentiated and polarized epithelial mutant cells no longer assembled an extracellular matrix (ECM), and there was no TIMP3 protein present in the ECM of mutant cells. Transmission electron microscopy revealed that mutant cells lacked extensive basal projections in seen in wild type cells, and confocal microscopy showed that these processes contained Collagen Type IV and laminin, suggesting that they might be an in vitro homologue of basal lamina infoldings, a structure with an important filtration function in the RPE in vivo. TIMP3 limits the action of metalloproteinases following tissue repair; we hypothesize if the protein cannot associate with the ECM, protease action continues, and ECM deposition cannot occur. Our findings are supported by other recent reports on in vitro models of other forms of macular degeneration, and by in vivo results in a mouse model of a related RPE disorder, butterfly shaped pigmentary macular dystrophy, caused by mutations in Ctnna1. Disruption of the basement membrane, leading to weakening and compromise of the epithelial barrier and opening the way to inflammation, immune attack, and neovascularization, may be a common early pathology in macular degeneration.

Funding Source: Supported by the Bright Focus Foundation and the Edward N. and Della L. Thome Memorial Foundation

Keywords: age related macular degeneration model, retinal pigment epithelium, extracellular matrix

Poster: 749

DEVELOPMENT OF AN RPE-SPECIFIC IN VITRO STARGARDT DISEASE MODEL

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The retinal pigment epithelium (RPE) is a polarized tissue that supports photoreceptors of the eye, transports nutrients, secretes growth factors and cytokines and is directly associated with vision. Our lab studied the surface proteome of RPE by cell surface capturing technology which revealed the presence of ABCA4 on the surface of RPE. We focused on the role of the ABCA4 protein as mutations in ABCA4 gene have been associated with various retinal degenerative diseases including the Stargardt disease. To specifically understand the function of ABCA4 protein in RPE, we developed an in-vitro Stargardt disease model using ABCA4-/- induced pluripotent stem cells (iPSC)-derived RPE. We used CRISPR-CAS9 technology to generate ABCA4-/- iPSCs which were differentiated into RPE. These RPEs were matured on transwells (semi-permeable membrane) for 6 weeks before analysis. The RPE monolayers were evaluated by structure, morphology and function by measuring the transepithelial resistance, electrophysiology and ability to digest photoreceptor outer segments. ABCA4 knockout was confirmed by RT qPCR, dd PCR, immunostaining and Sanger sequencing of the gDNA. RPE cells were treated with photoreceptor outer segments for a period of 8 days or by human complement for 48 hours and sub-cellular lipid deposits were evaluated in those cells by immunostaining to outline the sequence of pathogenesis of Stargardt disease. ABCA4-/- iPSC-RPE displayed normal electrophysiological response and structural properties compared to the ABCA4-WT iPSC-RPE. The knockout ABCA4 RPEs exhibited reduced ability to digest

photoreceptor outer segments and exhibited sub-cellular lipid accumulation while exposed to photoreceptor outer segment or human complement regimen. Our results indicate that ABCA4 plays an important role in RPE function and loss of ABCA4 leads to the functional defects in RPE cells contributing to Stargardt disease pathogenesis.

Keywords: induced pluripotent stem cell technology, Stargardt retinal degeneration, ABCA4 protein

Poster: 750

IN VITRO MODELING AND RESCUE OF CILIOPATHY ASSOCIATED WITH MUTATIONS IN NPHP5 USING PATIENT DERIVED CELLS

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Patient mutations in IQCB1 gene encoding the ciliary protein Nephrocystin 5 (NPHP5) cause early-onset retinal disease Leber congenital amaurosis (LCA) and kidney dysfunction in Senior-Loken syndrome. There is currently no treatment for NPHP5-LCA. In order to establish patient-specific in vitro disease models, we obtained dermal fibroblasts from a family affected by NPHP5-LCA, reprogrammed them into induced pluripotent stem cells and differentiated into ocular tissues: retinal organoids and retinal pigment epithelium (RPE). While ciliogenesis is retained in patient fibroblasts, RPE and retinal organoids, cilia morphology is affected with presence of abnormally elongated axonemes. Analysis of RPE cell cilia reveals accumulations of IFT88 and GLI2, suggesting disruption of intraflagellar trafficking and Sonic Hedgehog signaling in patient cilia. NPHP5-LCA patient retinal organoids show correct photoreceptor specification and expression of cell type-specific markers. However, development of outer segment-like structures, which are modified photoreceptor primary cilia, is impaired. Localization of Rhodopsin and cone L/M Opsin visual pigments to the apical outer segment-like region is reduced in NPHP5-LCA organoids. Importantly, this phenotype can be rescued by in vitro gene augmentation therapy using an AAV vector encoding correct NPHP5 sequence, providing a path for future treatment of NPHP5-LCA.

Funding Source: National Institutes of Health Intramural Research Program

Keywords: Retinal organoids, AAV gene therapy, Ciliopathy

Poster: 751

DEVELOPMENT OF AN IMPG2-RP MODEL USING HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL ORGANOIDS

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Retinitis pigmentosa (RP) is a heterogeneous group of rare inherited retinal degenerative diseases primarily characterized by loss of rod photoreceptors. Understanding the mechanisms of these genetic disorders can open potential avenues for treatment and shed light on basic molecular and cellular processes of vision. While animal models are an important tool for studying RP, disease phenotypes in these models are often poorly correlated with clinical outcomes of human RP patients. Human pluripotent stem cell (hPSC) “disease-in-a-dish” models are increasingly relied upon to corroborate animal model findings or fill the void when animal model systems are lacking. We employed a well-characterized 3D hPSC retinal organoid (RO) culture protocol to study photoreceptor-specific changes caused by mutations in the gene expressing Interphotoreceptor Matrix Proteoglycan 2 (IMPG2). Using patient-specific and genetically engineered hPSCs, we investigated protein and cellular-level perturbations to hPSC-RO development to model a particularly aggressive form of IMPG2-mediated childhood onset autosomal recessive RP (IMPG2 arRP). In vivo, IMPG2 is expressed by photoreceptors and is involved in promoting growth and maintenance of outer segments. ROs derived from hPSC lines harboring IMPG2 mutations fail to maintain photoreceptor outer segments relative to age-matched hPSC-RO controls. Furthermore, immunocytochemistry revealed that IMPG2 mutations in hPSC-ROs result in a complete lack of interphotoreceptor matrix proteins such as IMPG1. This provides a robust hPSC-RO platform in which to test and advance therapeutics for IMPG2 arRP.

Funding Source: Foundation Fighting Blindness Stem Cell and Regenerative Medicine Center-University of Wisconsin-Madison NIH grant U54HD090256

Keywords: Retina, Modeling, Organoids

Poster: 752

IPSC-DERIVED NK CELL DIFFERENTIATION REVEALS THE IMPACT OF CELL-CELL INTERACTIONS IN CELL SURVIVAL AND DEVELOPMENT

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Natural killer (NK) cells are innate lymphocytes that play a critical role in controlling viral infection and tumor immunity. Individuals with primary immunodeficiencies with a specific loss of NK cells have severe, recurrent viral infections and increased malignancy. Such NK cell deficiencies (NKDs) can be caused by mutations in DNA replicative helicase components, including MCM and GINS proteins. We hypothesized that the effects of these variants accumulate throughout hematopoietic development and maturation, making NK cells specifically vulnerable. To test this hypothesis, we generated and differentiated induced pluripotent stem cells (iPSCs) from an individual with NKD due to compound heterozygous GINS4 mutations and three of his family members who carry one or both of the damaging variants. First, CD34+ cells were produced from the embryoid body (EB) within 6 days of culture. When cultured on OP9 stromal cells, CD34+ cells from individuals with both damaging GINS4 variants failed to survive and generate mature NK cells, providing

recapitulation of the NK cell phenotype of the proband, whereas CD34+ precursors from other family members or unrelated controls generated functionally mature NK cells. Surveying the cells through the six-week-long differentiation showed defects in lineage specification at earlier time points, suggesting that NKD is a developmental phenotype. As an alternative to OP9 stromal cells, we differentiated the CD34+ cells with spontaneously generated stroma from the EBs, without sorting. In such culture, control CD34+ cells progressed to NK cells through similar steps and time points compared to the OP9 co-culture. GINS4 compound heterozygous lines again showed attenuated commitment to NK cells. However, by the end of the five weeks, mature NK cells were observed in these cell lines, albeit at different frequencies, suggesting stroma can change precursors' survival and potential to partially rescue NKD. Together, our work demonstrates that iPSC-derived differentiation recapitulates NKD phenotypes and provides a powerful system to investigate how the disease is shaped through differentiation. Usage of different co-culture and variant lines provides a new understanding of the properties of cell-cell interaction that derives NK cell development.

Funding Source: This work is supported by NYSTEM training grant and NIH-NIAID R01AI137275.

Keywords: Natural Killer cell deficiency, Differentiation, Cell-cell interaction

Poster: 753

INVESTIGATING THE ROLE OF THE AD PROTECTIVE CD33 VARIANT IN THE CONTEXT OF APOE GENOTYPE USING HUMAN IPSC DERIVED MICROGLIA

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Recent Genome Wide Association Studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) within genes expressed in microglia that regulate the late-onset Alzheimer's Disease (LOAD) risk. Inheritance of e4 allele of Apolipoprotein E (APOE4) or presence of R47H mutation in triggering receptor expressed on myeloid cells 2 (TREM2) increased risk of Alzheimer's Disease (AD), while the presence of rs12459419 T variant in CD33, a Siglec family transmembrane glycoprotein is protective. Given the genetic link between APOE4 and CD33 on LOAD and a strong correlation between the copy number of the protective CD33 rs12459419T allele with the dose-dependent decrease in AD risk, human induced pluripotent stem cells (iPSC)-derived microglia were generated to understand the synergistic contribution of these variants in the development of AD. Episomally reprogrammed iPSCs from (i) healthy donors expressing APOE3/3 genotype and (ii) AD donors expressing an APOE4/4 genotype with the CD33 protective rs12459419T allele or the non-protective rs12459419C allele were expanded and successfully differentiated into microglia. Cryopreserved microglia from all donor samples expressed microglia-specific cell markers (CD45, TREM2, CD33, P2RY12, TMEME119, CX3CR1, IBA-1). Functional assessment of end stage cryopreserved microglia displayed altered kinetics of phagocytosis and differences in soluble TREM2 levels between donors harboring either the protective rs12459419T or the rs12459419C allele. Microglia were treated with pro-inflammatory (M1) or anti-inflammatory (M2) stimuli to elucidate pathways involved during distinct phases of neural inflammation and neurorepair. Microglia derived from donors harboring the protective rs12459419T allele released higher levels of immunomodulatory M2 analytes including IL-10, IL-13, IL-12, IL-27, CCL8, CCL13 and CCL6 compared to microglia harboring the non-protective rs12459419C allele in donors harboring APOE 3/3 versus APOE4/4. These findings unveil the mechanism of the cellular responses elicited by the protective rs12459419T allele in the context of APOE genotype. This panel of iPSC-derived microglia can be used to understand the interplay of genetic variants involved AD risk and identify therapeutic targets for AD treatment.

Keywords: human iPSCs, Microglia, CD33, APOE, AD protective CD33 variant Vs APOE genotype, iPSC derived Microglia for disease modelling

Poster: 754

A BIOENGINEERED HUMAN LIVER MICROENVIRONMENT TO SUPPORT MOUSE HEMATOPOIETIC STEM CELL MAINTENANCE AND EXPANSION

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Rapid self-renewal and expansion of functional hematopoietic stem cells (HSCs) is a perennial goal of regenerative medicine. HSCs are the main therapeutic source to treat hematologic malignancies and disorders via bone marrow transplantation. Over 17,000 people require bone marrow transplants annually, according to the US Department of Health and Human Service (https://bloodcell.transplant.hrsa.gov). While HSCs have high therapeutic value, issues with graft-rejection from allogenic transplants and the inability to culture HSCs ex vivo are the current critical limitations. Hence, a system that can support the expansion of HSCs in vitro would greatly support further clinical applications of bone marrow transplants. While the bone marrow is the primary site of hematopoiesis, the fetal liver and adult liver are two other microenvironments that can support HSCs. 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 these hematopoietic microenvironments ex vivo. We have created a bioengineered coculture system that consists of primary human hepatocytes (PHHs) and 3T3-J2 mouse embryonic fibroblasts. Onto this coculture of PHHs and fibroblasts (PHH+J2), we cultured hematopoietic stem and progenitor cells (HSPCs) in serum-free medium supplemented with pro-hematopoietic cytokines such as stem cell factor (SCF) and thrombopoietin (TPO). HSPCs cultured on this PHH+J2 microenvironment for two weeks expanded over 200-fold 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 markers of CD48- and CD150+. In addition, the expanded cells were transplanted into lethally irradiated recipient mice to determine HSC functionality. The expanded cells from the PHH+J2 microenvironment engrafted and were able to

reconstitute the blood in serial transplantations. We further plan to expand functional human HSPCs ex vivo and understand the key niche factors that lead to HSPC maintenance and expansion.

Keywords: hematopoietic stem cell, hematopoietic stem cell expansion, liver microenvironment

Poster: 755

GLUTAMATERGIC DYSFUNCTION PRECEDES NEURON LOSS IN CEREBRAL ORGANOIDS WITH MAPT MUTATION

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Frontotemporal dementia (FTD) due to MAPT mutation causes pathological accumulation of tau and glutamatergic cortical neuronal death by unknown mechanisms. We used human induced pluripotent stem cell (iPSC)-derived cerebral organoids expressing tau-V337M and isogenic corrected controls to discover early alterations due to the mutation that precede neurodegeneration. At 2 months, mutant organoids show upregulated expression of MAPT, and glutamatergic signaling pathways and regulators including the RNA-binding protein ELAVL4. Over the following 4 months, mutant organoids accumulate splicing changes, disruption of autophagy function and build-up of tau and P-tau S396. By 6 months, tau-V337M organoids show specific loss of glutamatergic neurons of layers affected in patients. Mutant neurons are susceptible to glutamate toxicity which was rescued pharmacologically by treatment with the PIKFYVE kinase inhibitor apilimod. Our results demonstrate a sequence of events that precede cell death, revealing molecular pathways associated with glutamate signaling as potential targets for therapeutic intervention in FTD.

Keywords: Organoids, Frontotemporal dementia, Glutamatergic neurons

Poster: 756

ANALYSIS OF DISTINCT CELL POPULATION FATE IN PATIENT-DERIVED CEREBRAL ORGANOID MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune neurological disorder characterized by inflammation, demyelination and neural degeneration. However, the origin and evolution of the disease are still poorly understood due to relative inaccessibility of human brain tissue and inadequate animal models to study MS. Recent advances in 3D cerebral organoid cultures derived from induced pluripotent stem cells (iPSC) provide new avenues to develop models to study cell type- and stage-specific effects of MS. Cerebral organoids contain ventricle-like structures aligned by neural stem cells, progenitor cells in various stages of differentiation and migration, and cortical neurons in a stereotypical inside-out stratified layout. Moreover, it has been previously shown that neurons present in c-organoids were able to get myelinated. We propose here to develop an innovative model of MS using human cerebral organoids derived from iPS cells of patients with MS. Stem cell proliferation, migration and differentiation in neuronal and glial lineage were assessed in the different type of MS organoids and compared to control organoids after 42 days in vitro. Upper layers of the cortical structure in PPMS c-organoids were larger compared to control c-organoids, suggesting an imbalance of the stem cell proliferation/differentiation capacity. Immunofluorescence staining for stem cell marker SOX2 revealed that the stem cell pool, localized in the VZ, was significantly reduced in MS organoids compared to control. Additionally, a staining of neuronal markers CTIP2 and TBR1 highlighted an increase of neurogenesis, in PPMS particularly, compared to control. To explain this disturbance of the proliferation/differentiation capacity of the stem cell pool, an analysis of the cleavage plane angle of mitotic cells was performed. A shift from symmetric division towards asymmetric division was revealed in MS compared to control, suggesting an increase of stem cell differentiation and neurogenesis in spite of stem cell proliferation. Further investigations are needed to understand the mechanisms involved. This study describes an innovative model of MS and will give new insights on the origin and evolution of the disease and will help to identify potential targets for therapeutic strategies in the different types of MS.

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

Keywords: Multiple sclerosis, Cerebral organoids, IPS cells

Poster: 757

IDENTIFICATION OF ARC EXPRESSION IN INDUCED PLURIPOTENT STEM CELLS (IPSCS)

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Arc (Activity-Regulated Cytoskeleton-Associated Protein; Arg3.1) is an early immediate gene with a central role in synaptic plasticity and cognition. Dysregulation of Arc and associated networks are implicated in numerous neurological disorders, such as schizophrenia, autism spectrum disorder, and Alzheimer's disease. Arc has largely been studied in neurons, where it is rapidly upregulated in response to neuronal activity and is trafficked from the nucleus to sites of synaptic activity. While Arc has also been detected in a few other tissues outside the brain and has been shown to increase in response to growth factors and cellular stress, the function of Arc outside of the nervous system remains poorly understood. We examined Arc in human induced pluripotent stem cells (iPSCs) and discovered that Arc is constitutively expressed at high levels in iPSCs and localizes as punctate structures outside of the nucleus, consistent with our data in human neurons. While it is unclear how Arc expression is regulated in the iPSCs, we found that baseline expression of Arc declines during iPSC differentiation along the neuronal lineage.

Our studies aim to elucidate the biological significance of Arc expression in iPSCs to gain a mechanistic understanding of Arc function in pluripotent stem cells and in tissue outside of the nervous system.

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Keywords: hIPSC, Neurodevelopment, IEG

Poster: 758

MODELING SARS-COV-2 INFECTION OF THE BRAIN USING HUMAN CORTICAL ORGANOIDS

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The prevalence of neuropsychiatric disorders is increasing, constituting approximately 28% of the global burden of non-communicable disease. A major cause of these disorders is that of aberrant fetal brain development, and there is growing evidence implicating prenatal exposures as a driving factor. A common exposure that has undergone an exponential increase in incidence during recent years is that of viral infections, due in part to the novel coronavirus disease 2019 (COVID-19) pandemic. Up to 1/3 of COVID-19 patients have manifested neurological complications, and RNA from the etiologic agent SARS-CoV-2 has been found in brain biopsies from fatal cases. Furthermore, increasing rates of pre-term birth, miscarriages, and other defects have been seen following a maternal infection. The neurotropism of SARS-CoV-2 and the implications of neuroinflammation on both the adult and developing brain are still poorly understood. Therefore, it is imperative to gain insight into the mechanism(s) responsible for these complications. We hypothesize that exposure to SARS-CoV-2 and pro-inflammatory cytokines triggers neurodevelopmental changes leading to a disruption of neural structure and circuit function in the developing cortex. Due to the inaccessibility of brain tissue from COVID-19 patients, we will address these questions using human stem cell-derived cortical organoids. To elucidate the cellular and molecular effects of prenatal COVID-19 exposure on the development of the fetal brain, we will first examine the neurotropism of SARS-CoV-2 in cortical organoids by identifying the cell types susceptible to a productive SARS-CoV-2 infection, and evaluating the consequential effects on infected cells for Aim 1. For Aim 2, we will determine the mechanism by which neuroinflammation influences SARS-CoV-2 infection in cortical organoids using immunostaining and functional assays to evaluate alterations in viral infection levels, viral replication, cellular integrity, and neuronal networks. This study aims to reveal novel insight into the specific cellular and molecular etiology of potential neuropsychiatric disorder development from prenatal COVID-19 exposure, potentially contributing to future therapies and interventions.

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Keywords: SARS-CoV-2, Organoids, COVID-19

Poster: 759

ASSESSING MOLECULAR SIMILARITIES IN HESC MODELS OF STRIATAL NEURONS

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Dynamic molecular changes in medium spiny neurons (MSN) of the striatum underlie brain diseases including drug addiction. Many of these molecular mechanisms have been well established in rodent brains. However, little is known about the molecular dynamics of human MSNs in response to perturbations of interest due to ethical limitations and experimental tractability. We propose a reductionist human embryonic stem cell derived (hESC) model of MSNs that can be perturbed and analyzed with molecular precision. We have differentiated hESC to DARPP32+/GABA+/CTIP2+ neurons that capture, at least in part, the molecular dynamics of striatal MSNs. In this work, we evaluated hESC-derived MSNs for transcriptional and functional responses to acute and chronic dosing regimens of dopamine. The transcriptional response after dopamine exposure resembled rodent responses to cocaine, specifically the upregulation of immediate early genes (FOS, FOSB, JUNB, ARC). FOSB was expressed ~50% more than ΔFOSB, as was expected. THBS1, GRIA3, PPP1R1B, and TAC1 were differentially regulated indicating the induction of synaptic plasticity, glutamate receptor, and phosphatase genes, respectively, in response to dopamine dosage. Overall, the transcriptional profile shifted after chronic dosing, showing a ~50% reduction in the number of differentially expressed genes compared to acutely dosed MSNs relative to vehicle controls. Ingenuity pathway analysis of differentially expressed genes from each condition showed shared pathways (NRF2-mediated oxidative stress response) and pathways unique only to acute or chronic conditions (Dopamine-DARPP32 Feedback in cAMP Signaling, EIF2 Signaling) as top pathways, in accordance with previous rodent studies. Furthermore, we observed gene desensitization in chronically dosed samples in a subset of genes (NPAS4, DKK1) implicated in development and long-term memory. Finally, functional assays show a similar desensitization exhibiting reduced frequency and amplitude of calcium transients an hour after dopamine dosing. The work presented here highlights the early potential for developing a reductionist approach to complement rodent work in dissecting human molecular mechanisms related to the striatum and addiction.

Funding Source: This work was supported an NIH Avenir Award (DP1-DA044359).

Keywords: medium spiny neuron, dopamine, RNAseq

Poster: 760

AUTS2 FORMS A NEURONAL PROGENITOR-SPECIFIC COMPLEX WITH SKI TO REGULATE NEURONAL DIFFERENTIATION THROUGH CUL4-MEDIATED INHIBITION OF BMP PATHWAY

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Neurodevelopmental disorders (NDD) result from compromised development of the central nervous system and encompass but are not limited to Autism Spectrum Disorders (ASD), Developmental Delay (DD) and Intellectual Disabilities (ID). Recently, Autism Susceptibility Candidate 2 (AUTS2) has been identified as one of most frequently disrupted genes in NDD. Disruption of AUTS2 leads to common neurodevelopmental abnormalities including microcephaly, developmental delay, and varying degrees of intellectual disability. The importance of AUTS2 in neurodevelopment has been confirmed using animal models and embryonic stem cells (ESC). However, the mechanisms by which AUTS2 regulates neuronal fate at the molecular level remain unclear. Our previous studies have shown that nuclear AUTS2 comprises a type 1 Polycomb repressive complex (PRC1) and converts PRC1 from transcriptional repressors to activators. However, cytosolic AUTS2 seems to be crucial for neuronal migration and neuritogenesis in mouse cortex through the activation of Rac1. Additional factors that interact with AUTS2 also have been identified and likely play roles in neurodevelopment. In an unpublished study, using mouse ESCs with Auts2 loss of function engineered by CRISPR/Cas9 editing as well as primary cortical neurons isolated from Auts2 knockout mice, we discovered that Auts2 engages the BMP pathway through a novel mechanism to promote neuronal differentiation. Transcriptome analysis revealed that Auts2 deficient mouse ESCs displayed pronounced defects in neuronal differentiation with concomitant up-regulation of BMP signaling. This phenotype was further confirmed in cortical neurons with Auts2 deletion. Mechanistically, with quantitative mass spectrometry analysis, we identified a novel protein complex comprised of AUTS2, WDR68 and SKI (the AWS complex), which is specifically formed in neuronal progenitors and mediates the inhibition of BMP signaling during differentiation. Furthermore, biochemical characterization revealed that the AWS complex recruits the CUL4 E3 complex to restrict the availability of BMP-specific regulatory SMAD1/5/9 through ubiquitination and proteasomal degradation. These findings will likely provide new directions for the development of effective therapeutic interventions in NDD.

Keywords: AUTS2, Neuronal differentiation, BMP pathway

Poster: 761

MODELING FAMILIAL ALZHEIMER'S WITH HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL SPHEROIDS

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Alzheimer's Disease (AD), the most common neurodegenerative disorder, is the only disease of the top ten most prominent in the United States that has seen significant increase in the number of affected people. There are currently no treatments that stop or prevent the onset of AD as the underlying mechanisms causing AD have not been determined. Genetic mutations in the presenilin1 gene (PSEN1) account for over 80% of all familial AD (fAD) cases. Although over 300 of these mutations have been reported, the mechanism by which these mutations cause fAD are still unclear. As the catalytic component of the gamma secretase, PSEN1 can have pleiotropic effects in the brain. Buildup of amyloid plaques are seen in all AD brains and are a result of PSEN1-mediated cleavage of the amyloid precursor protein (APP). PSEN1 also plays a large role in brain development through the Notch signaling pathway which governs cell fate decisions during neurogenesis. Our central hypothesis is PSEN1 mutations affect Notch signaling, APP processing, or both, to cause fAD. While there are many mouse models available for studying this gene, they fail to recapitulate the pathology and behavior seen in fAD and require several mutations to be induced and overexpressed in order to see pathological changes. Therefore, there is a need for a human-based model to study the cellular mechanisms that cause fAD as a direct result of mutations in PSEN1. Three dimensional human cortical spheroids (hCS) derived from patient induced pluripotent stem cells (iPSCs) are a valuable tool that can be used to study the effects of these genetic mutations in the human brain during development and disease. We have created hCS from three different CRISPR edited iPSC lines carrying genetic mutations in the PSEN1 gene, L435F, M146L, and D385A, that have been found in patients with fAD. We will be determining the effects of genetic mutations in PSEN1 in the Notch signaling pathway and subsequent proliferation, differentiation and cell death during the development of hCS. We will also be determining how mutations in PSEN1 affect APP processing and cause fAD-associated neuropathology. Together, the results of these experiments can give insight into how PSEN1 causes cellular changes in the brain to cause fAD and provide targets for future treatments to stop or reverse the progression of AD.

Keywords: Presenilin1, Alzheimer's, spheroids

Poster: 762

DIFFERENTIATIOM OF HUMAN PLURIPOTENT STEM CELLS INTO RADIAL GLIA AND ASTROCYTES BYPASSES NEUROGENESIS

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Human brain development starts with the formation of the neural tube followed by the emergence of radial glial cells (RGC) that act as neural stem cells. Typically, RGC will yield neurons first and then glial cells at later stages. Therefore, the molecular mechanisms that control RGC differentiation into astrocytes during late brain development remain poorly understood. Hence, derivation of astrocytes from induced pluripotent stem cells (iPSCs) has been inefficient, variable, and long-lasting, reliant on the use of xenogeneic material or forced expression of transcription factors. Here, we developed a highly efficient and chemically defined astrocyte differentiation strategy that requires neither serum nor genetic manipulation. Notably, this approach bypasses neurogenesis, which otherwise always precedes astrogliogenesis during brain development. iPSCs were first differentiated into RGC-like cells in 7 days and, unlike the dual-SMAD inhibition strategy, these neuralized cells exhibited in vivo-like radial glia signatures. Activation of gliogenic cell signaling pathways in RGCs then resulted in direct astrogliogenesis demonstrated by strong upregulation of SOX9/NFIA/NFIB and NFIX gliogenic transcription factors throughout the differentiation process and expression of CD44/S100B/SLC1A2 by day 30, followed by GFAP expression. Time-course chromatin analysis (ATAC-seq) confirmed multiple accessible GFAP and AQP4 domains. Calcium imaging, cytokine response, co-culture experiments with neuronal cells, and grafting into mouse brains confirmed the specific identity and functionality of iPSC-derived astrocytes. As an example for disease modeling, iPSCs from a patient with Alexander disease were differentiated into astrocytes and previously reported cellular pathologies (e.g. GFAP aggregates) as well as new disease phenotypes were identified. In conclusion, our results shed new light on cell fate specification by identifying remarkable plasticity in neural lineage progression that can be exploited to manufacture large numbers of iPSC-derived astrocytes for disease modeling, drug screening and regenerative medicine.

Keywords: Radial Glia, Astrocytes, iPSC

Poster: 763

TRANSCRIPTOMIC ANALYSIS IDENTIFIES WNT/BETA-CATENIN SIGNALING AS A POTENT MITOGEN THAT CAN EXPAND ESC CULTURES TO OBTAIN HIGHER YIELDS OF SPINAL SENSORY INTERNEURONS IN VITRO

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Distinct populations of dorsal interneurons (INs) in the spinal cord mediate different sensory modalities, including pain, itch, heat, and balance. These neurons are often damaged in spinal cord injuries (SCI), such that regenerative therapies to recover somatosensation in SCI patients will require the de novo generation of large numbers of function-specific INs. Towards this goal, we have developed the first directed differentiation protocols to produce sensory modality-specific INs from mouse and human embryonic stem cells (m/hESCs). The addition of two growth factors - retinoic acid (RA) and Bone Morphogenetic Protein 4 (BMP4) - in a tightly controlled time window, is sufficient to direct ESCs towards the full complement of sensory INs. Single-cell RNA-Seq analyses have identified that mESC-derived INs display the relevant modality-specific transcriptomic modules and are indistinguishable from the transcriptomic profiles of sensory INs isolated from mouse embryos. Thus, the ESC-derived INs faithfully replicate the status of endogenous dorsal INs. However, while our protocols generate different sensory INs with 10-20% efficiency, expansion capacity has been limited. In vitro-derived spinal dorsal progenitors die after multiple passages, making it challenging to generate the large numbers of sensory INs needed for clinical and pharmacological uses. To resolve this issue, we have identified that the Wnt family is a potent mitogen for ESC-derived dorsal sensory progenitors. Treating ESCs with a Wnt agonist increases proliferation by ~10 fold and sustains dorsal progenitor proliferation for multiple passages. Greatly increased numbers of modality-specific sensory INs can then arise when Wnt signaling is depleted, in combination with the inhibition of Notch signaling. Taken together, this study identifies the methods to generate bona fide sensory INs in the large numbers required for either regenerative therapies or drug discovery platforms.

Funding Source: Broad Stem Cell Research Center, UCLA NINDS/NIH (NS085097) CIRM (RB5-07320)

Keywords: Sensory interneurons, Wnt signaling, mouse and human embryonic stem cells

Poster: 764

MICROGLIA STATE IS MODIFIED BY APOE E4 GENOTYPE, INHIBITING SURVEILLANCE OF NEURONAL-NETWORK ACTIVITY AND IMPAIRING SYNAPTIC REMODELING

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Altered neuronal excitability has emerged as a common pathological feature observed across many patients and animal models of Alzheimer's Disease (AD). Elevated neuronal activity may exacerbate disease pathology, as patients with AD and seizure disorders exhibit cognitive decline several years earlier than AD patients without seizure disorders. Although microglia are known to sculpt and modulate neuronal circuits, our understanding of the role of these cells to network-level deficits observed in AD remain unclear. In this study, we derive forebrain organoids and microglia-like cells from CRISPR-edited APOE e3/3 (APOE3) and e4/4 (APOE4) isogenic iPSC lines with the goal of defining the impact of microglia on neuronal network activity. By modulating neuronal activity in forebrain organoids, we first observed that microglia-like cells respond by displaying calcium transients in a neuronal activity-dependent manner, while APOE4 microglia are only weakly attuned to neuronal activity. This deficit was attributed to functional differences in purinergic signaling mediated by an altered microglia state, which we define at multiple molecular and functional levels. While APOE3 excitatory neuronal networks display highly coordinated ensemble events, APOE4 neurons were hyperexcitable and highly uncoordinated. We interrogated the APOE-genotype dependent contribution of microglia to modulating neuronal excitability through combinatorial experiments and found distinct and opposing effects of APOE3 and APOE4 microglia on network excitability. Our work underscores the ability to model neuron-microglia cross talk using IPS-based models for dissecting the contribution of AD risk genes to neuronal network disfunction.

Funding Source: HHMI Hanna H. Gray Postdoctoral Fellowship

Keywords: Forebrain Organoids, Microglia, APOE, GCaMP, Neural Networks

Poster: 765

MODELING MACROCEPHALY IN AUTISM SPECTRUM DISORDER USING HUMAN IPSC-DERIVED NEURAL PRECURSORS AND BRAIN ORGANOIDS

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, displaying a vast range of phenotypes. Its prevalence has drastically increased over the years, with ASD now being the fastest growing neurodevelopmental disorder. Due to its complexity, untangling the underlying mechanisms and identifying therapeutic targets have proven challenging. The use of induced pluripotent stem cell (iPSC) technology allows generation of in vitro models of ASD which combined with clinical data, have the potential to revolutionize this field of research. Macrocephaly (disproportionate brain enlargement) has consistently been reported in 15-20% of ASD patients. Furthermore, macrocephaly often precedes clinical symptoms of ASD and it is generally associated with more severe behavioural and cognitive symptoms. Understanding this association and the underlying mechanisms that regulate such changes could uncover potential therapeutic targets and provide a window of opportunity for intervention and symptom mitigation. In this study, human iPSC-derived neural precursor cells (NPCs) and brain organoids from ASD patients, with and without macrocephaly, were generated and compared to healthy controls. We then explored whether changes in cell proliferation and phagocytosis regulate brain size and contribute to the macrocephaly phenotype. Data suggests that differences in neuronal proliferation pathways mechanistically converge with neuroimmune mechanisms. To identify how these signaling mechanisms play a role in cell survival and clearance, we co-cultured our iPSC-derived NPCs and brain organoids with immune-derived cells and assessed differences in cellular elimination. In summary, we propose that targeting the neuroimmune system could be important to improve diagnosis, prognosis and establish possible targets for clinical intervention and symptom mitigation in ASD patients with macrocephaly.

Keywords: Brain Development, ASD, Organoids

Poster: 766

ALTERED ADULT NEUROGENESIS IN HUMAN MESIAL TEMPORAL LOBE EPILEPSY

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The hippocampus is the most common seizure focus in people. Within the hippocampus, aberrant neurogenesis plays a critical role in the initiation and progression of epilepsy in rodent models, but it is unknown whether this also holds true in humans. The presence of adult neurogenesis in the human hippocampus has been contentious in recent years. In this study, we used immunofluorescence on control hippocampus and surgical resections from mesial temporal lobe epilepsy (MTLE), neurosphere cultures, and multi-electrode recordings of patient ex vivo hippocampal slices. We found that a longer duration of epilepsy is associated with a sharp decline in neuronal production, but no detected change in astrogenesis. Further, immature neurons are mostly inactive, and are not observed in cases with local epileptiform activity. However, immature astroglia are present in every MTLE case and their location and activity are dependent upon epileptiform activity. Immature

astroglia, rather than newborn neurons, therefore represent a new target for therapeutic development to modulate adult human neuronal hyperactivity.

Keywords: human neurogenesis, neural stem cell, disease

Poster: 767

COMPARATIVE TRANSCRIPTOME PROFILING OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NOCICEPTORS TO HUMAN DORSAL ROOT GANGLION TISSUES

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The scarce availability and prohibitive costs of primary human dorsal root ganglion (hDRG) tissues limits the scalability of in vitro models for pain drug discovery and disease modeling. Human induced pluripotent stem cell (hiPSC) derived sensory neurons provide a near-limitless amount of tissue for these applications. Using an accelerated, step-wise, directed differentiation protocol that proceeds through a novel 'primal ectoderm' population, we are able to manufacture nociceptors rapidly and at scale by relying on a simplified chemically-defined basal medium, recombinant growth factors and small molecules. Following production, we matured the nociceptors over a period of four weeks and performed RNA sequencing at weekly time points to compare with publicly available hDRG tissues data repositories. These cells transition through a conserved developmental regulatory program that includes NEUROG1/2, NEUROD1/2, and ISL1/2. Upon maturation, they show stable expression of hallmark nociceptor genes including TRKA, CALCA, and P2RX3. Robust expression was observed for 64 out of 80 conserved DRG markers, with the noticeable exception of myelinating factors, highlighting a pure population of neurons. We correlate this molecular data with time-course whole-cell patch clamp electrophysiology studies that show the progressive maturation of these neurons to DRG-specific voltage gated sodium ion channels, action potentials and bust-firing ability. Together, these data demonstrate that hiPSC nociceptors progress through a robust directed differentiation protocol and have the potential to screen and identify novel analgesic or anti-nociceptive compounds for use in humans.

Keywords: pain drug discovery, DRG, nociceptors

Poster: 768

MONITORING ORGANOID DEVELOPMENT AND CHARACTERIZATION OF CALCIUM OSCILLATION ACTIVITIES IN IPSC-DERIVED 3D CEREBRAL ORGANOIDS

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Cerebral organoids are a rapidly developing technology that has great potential for understanding brain development and neuronal diseases, and can also be used for testing effects of compounds and genetic mutations. The model allows characterization of later events in cortical development, which provides an advanced and more biologically relevant system for research and drug discovery. Further method development would include adoption of the model for compound screening, and testing the functional neuronal activities. We describe the methods for monitoring cerebral organoids and testing their functional activity by recording and analyzing calcium oscillations. Cerebral organoids were developed from iPSC using established methods. We monitored size and morphology of developing brain microtissues over 6-8 weeks of development, using AI-based analysis tools for defining size, shape, and density of the tissues. Selected microtissues were analyzed by confocal imaging during different phases of development for cell organization and expression of neuronal markers. For detection of functional activities, organoids were loaded with calcium sensitive dye and then Ca2+ oscillations were recorded with the IXM-C Imaging System or the FLIPR System. Calcium oscillations from individual cell clusters, or entire organoids, were recorded and analyzed with peak analysis software. Complex analysis of calcium oscillations allowed multiparametric characterization of oscillation patterns that included the oscillation rate, peak width and amplitude, secondary peaks, waveform irregularities, and multiple other readouts. Cellular and mitochondrial toxicity were monitored using live fluorescence imaging. For assay characterization, we used a set of neuromodulators affecting GABA, NMDA, and dopamine targets. Also we tested several neurotoxic substances. The observed changes in oscillation patterns were consistent with expected mechanism of actions of compounds. Modulations of calcium activity patterns and concentration dependencies of the effects were characterized across multiple readouts. Advanced biological system of 3D cerebral organoids paired with high-content imaging and complex analysis of calcium oscillations demonstrates a promising tool for testing compounds and toxicity evaluation.

Funding Source: No external funding

Keywords: brain organoids, calcium oscillations, high content imaging of organoids

Poster: 769

INVESTIGATING THE ROLES OF STORE-OPERATED CALCIUM ENTRY DURING DEVELOPMENT OF THE HUMAN AND MOUSE CEREBRAL CORTEX

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In the developing cerebral cortex, agonist-induced calcium waves, mediated in part by intracellular calcium stores, propagate through the germinal zones to modulate neural stem/progenitor cell (NSPC) proliferation. How internal calcium stores are maintained in cortical NSPCs and the mechanisms by which calcium dynamics regulate NSPC behavior, however, remain unclear. Here we begin to tackle this fundamental question by interrogating how store operated calcium entry (SOCE), a mode of calcium influx tied to depletion of endoplasmic reticulum (ER) calcium stores and implicated in neural progenitor proliferation in vitro, regulates calcium signaling in cortical NSPCs to influence their function and output. We have found that SOCE is required in both mouse and human primary fetal cortical NSPCs

to maintain proliferation. We have also found that two splice isoforms with opposing functions of the ER calcium sensor STIM2 are dynamically expressed during neuronal differentiation, such that an inhibitory isoform of STIM2 that suppresses SOCE is upregulated in young neurons. This result suggests that alternative splicing of STIM2 is a key regulator of SOCE in differentiating cells and is in line with our observation that robust SOCE responses in proliferative NSPCs are significantly diminished upon cell cycle exit. Further supporting this idea, we have found that manipulating the levels of STIM2 variants in vivo in embryonic mice using electroporation bidirectionally regulates cell cycle exit of cortical NSPCs. Our data suggests that dynamic regulation of SOCE mediators and downstream calcium signaling plays an indispensable role in the control of progenitor function in the developing cortex. As aberrant calcium signaling has been implicated in neurodevelopmental disorders, our studies defining the molecules and mechanisms involved in transducing calcium signals during cortical development will provide essential insights into normal and dysfunctional corticogenesis.

Keywords: neural development, calcium, neural stem/progenitor cell

Poster: 770

UNRAVELING THE ROLE OF STAU2 IN C9ORF72-ASSOCIATED PATHOLOGY USING HUMAN IPSC-DERIVED CORTICAL NEURONS

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A hexanucleotide (GGGGCC)n repeat expansion in the noncoding region of C9ORF72 is the most common pathogenic mutation associated with both frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). In C9ORF72 FTD patient cortical tissue, toxic dipeptide repeat proteins (DPR), polyGR and polyPR were found to colocalize with a RNA-binding protein (RBP), STAU2 in large granular structures but not with classical stress granule markers, pointing to a specific role for STAU2 in C9ORF72 aggregation. STAU2 is known to have roles in RNA localization, transport, stability and translation. Here we are using C9ORF72 ALS/FTD iPSCs to examine the potential role of STAU2 in disease course. To date, C9ORF72 iPSC-derived motor neurons have shown several disease-related features including compromised mitochondrial function, impaired nucleocytoplasmic transport, increased ER stress, proteosomal inhibition, and susceptibility to glutamate toxicity. Further, we found using spinal motor neurons derived from C9ORF72 ALS/FTD iPSCs, that STAU2 colocalizes with polyGR and polyPR aggregates in nuclear and cytoplasmic granules in patient but not control lines. Using functionally mature cortical neurons generated from multiple control and C9ORF72 ALS/FTD patient iPSCs, we show by immunostaining that C9ORF72 is expressed in both neural progenitors and differentiated neurons. After performing RNA-binding protein immunoprecipitation (RIP), we found the C9ORF72 transcript in the STAU2 cargo. In the presence of oxidative stress induced by sodium arsenite, we observed C9ORF72 protein aggregates in cytoplasmic STAU2+ granules. Currently, we are analyzing, in stressed and non-stressed iPSC-derived cortical neurons, the expression patterns of STAU2 and C9ORF72 in control and mutant lines, and the binding of normal and repeat-containing transcripts to STAU2. Moreover, we are examining the effect of overexpression of C9ORF72 polyGR/PR in a STAU2 knockdown line generated through CRISPR interference (CRISPRi). STAU2 expression is biased toward dendrites of cortical glutamatergic neurons, including those neuronal populations affected in FTD. Improved understanding of the interactions between STAU2 and C9ORF72 in both normal and disease contexts could provide new avenues to combat FTD and ALS.

Keywords: RNA-binding protein, C9orf72, iPSC-derived neurons

Poster: 771

STUDYING GNB1 DISORDER AT THE LEVEL OF THE PATIENT AND THEIR MUTATIONS

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GNB1 disorder is a rare neurodevelopmental disorder characterized by global developmental delay, intellectual disability, hypotonia, and seizures. It presents in individuals heterozygous for a mutant copy of the GNB1 gene, which encodes the Gβ1 subunit of heterotrimeric G proteins. Over 30 different de novo germline GNB1 mutations have been identified to date, yet little is known regarding how these mutations lead to the pathophysiology of GNB1 disorder. As such, we aim to better characterize aspects of the disorder by connecting functional outcomes to clinical phenotypes in a patient-relevant manner. In collaboration with the Montreal Neurological Institute's iPSC-CRISPR platform and Clinical Biological Imaging and Genetic Repository (C-BIGR), we are building a pipeline where GNB1 families can donate blood samples for the generation of patient-derived iPSC lines and CRISPR-corrected isogenic control lines. We have already generated an iPSC line from a GNB1 patient and confirmed the presence of the M101V mutation. Using these cell lines, we aim to characterize phenotypes during differentiation into cortical neurons by assessing features such as neural progenitor cell proliferation rates, neuronal maturity, and proportion of neurons to astrocytes. We then aim to connect these phenotypes to functional outcomes using a library of biosensors to study signalling via protein kinases A, B (AKT), and C, and ERK1/2, respectively. Since Gβγ can also interact with inwardly rectifying potassium channels and voltage-dependent calcium channels, we plan to use the GINKO1 and GCaMP biosensors and patch clamp electrophysiology to these channels as well. This project aims to expand our knowledge of GNB1 disorder by understanding affected signaling pathways and how they lead to neurological phenotypes observed. Understanding their impact could guide personalized therapeutic strategies that might help GNB1 children.

Funding Source: This work was funded by CIHR

Keywords: GNB1, neurodevelopmental disorders, cellular signaling

Poster: 772

BMP INHIBITION ALLEVIATES DEFECTS IN NEURAL DIFFERENTIATION CAUSED BY SMARCA2 MUTATIONS FOUND IN NICOLAIDES-BARAITSER PATIENTS

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The SWI/SNF complexes consist of up to fifteen subunits which are encoded by 29 genes. They contain an ATPase subunit, either SMARCA4 or SMARCA2, and utilize the energy from ATP hydrolysis to remodel chromatin structure by sliding and evicting nucleosomes to regulate gene expression. During the neural differentiation, SMARCA4 levels decline while SMARCA2 increases. However, the role of SMARCA2 in this process still remains poorly understood. Heterozygous mutations in the subunit SMARCA2 cause Nicolaides-Baraitser syndrome (NCBRS), a developmental disorder associated with intellectual disability. Previously, using human embryonic stem cells (hESCs), we reported that introduction of NCBRS-associated K755R and R1159Q SMARCA2 mutations caused retargeting of SMARCA4, which resulted in enhancer reprogramming that ultimately altered impaired neural differentiation. Specifically, SMARCA2 mutant neural progenitor cells (NPCs) skew toward astrogliogenesis during neural differentiation. It is known that Noggin, a BMP inhibitor, enhances neural differentiation by suppressing differentiation to other germ layers and the astrocytic lineage. Here, we hypothesized that BMP inhibition by adding LDN-193189 (LDN), a synthetic analog of Noggin, will alleviate the neural differentiation defects caused by SMARCA2 mutations. We found both the morphology and proliferation defects of SMARCA2 K755R and R1159Q mutant NPCs were rescued with LDN treatment. LDN-treated SMARCA2 mutant NPCs regained the expression of key NPC markers such as SOX2 and PAX6 and normalized overexpression of astrogliogeneic promoting factors including NFI and AP-1 transcription factors. Further, LDN-treated SMARCA2 mutant NPCs gave rise to mature neurons expressing SYN1 and MAP2. However, functional genomic experiments showed that Polycomb-dependent gene repression is dysregulated in LDN treated SMARCA2 mutant NPCs in that H3K27me3 at bivalent promoters of neural genes fails to resolve during neural differentiation, causing repression of a number of neural factors in SMARCA2 mutant NPCs. Overall, our data showed that inhibition of BMP signaling pathway during neural differentiation partially rescues the defects caused by NCBRS-associated SMARCA2 mutations and reveals underlying gene expression changes associated with disease.

Funding Source: CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375

Keywords: SWI/SNF complexes, SMARCA2, Epigenetic

Poster: 773

GENERATION OF HUMAN CORTICO-STRIATAL ASSEMBLOIDS TO STUDY HUMAN NEURAL CIRCUITS DEVELOPMENT AND DISEASE

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Cortico-striatal projections in the forebrain are critical components of cortico-basal ganglia circuits that regulate motivated behaviors. A major challenge in understanding the developmental assembly of this neural circuit and how its dysfunction leads to neuropsychiatric disease is the lack of access to functional human brain tissue. Here, we describe the derivation of three-dimensional (3D) striatal organoids from human pluripotent stem cells and show how they can be assembled with cortical organoids to form cortico-striatal circuits. To probe and manipulate human cortico-striatal circuits in vitro, we implemented a series of neuroscience tools, including rabies tracing of connections and optogenetics manipulation coupled with recording of neuronal activity with genetically encoded calcium indicators. These methods in combination with electrophysiology in assembloid slices demonstrated the assembly of functional human cortico-striatal circuits. Moreover, we identified disease-related cellular defects in cortico-striatal assembloids derived from patients with a neurodevelopmental disorder caused by a deletion on chromosome 22q13.3. We anticipate that this novel approach can be used to study human circuit assembly and to model disease.

Keywords: Brain assembloids, Brain organoids, 22q13.3 deletion syndrome

Poster: 774

POST-MITOTIC SENESCENT PHENOTYPES IN AGED HUMAN NEURONS FROM ALZHEIMER'S PATIENTS

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In vitro modeling of age-related diseases in humans has been confounded largely by iPSC-based protocols, which represent an embryonic intermediate where many signatures of aging are lost. Neurons directly induced from patient fibroblasts (iNs) retain the molecular and epigenetic age of their donor. Thus, iNs present an attractive model for studying the effects of age in neurons during healthy and pathological aging. Cellular senescence, initially described as replicative exhaustion of dividing fibroblasts in vitro, has had many of its components discovered in post-mitotic, non-diving cells types. The emergence of a senescence-like (SL) phenotype in post-mitotic cells typically occurs late in life, further linking senescence pathways to age even in cells that don't experience telomere shortening by cellular division. The extent to which senescence is initiated in human neurons

and the consequences of senescence induction have not been thoroughly evaluated, however, due to the inaccessible nature of aged human neurons. Using iNs, we have observed endogenous and age dependent activation of senescence pathways in human neurons. Furthermore, with a cohort of iNs derived from Alzheimer's disease (AD) patients and healthy age matched controls, we have observed significant upregulation of senescence genes, senescent epigenetic modifications, and increased senescent cell abundance in aged neurons of AD patients. While SL neurons constitute a minority of the total population, they could be a potent source of the chronic inflammation characteristic of the AD brain. Commensurately, we have detected elevated levels of pro-inflammatory factors in the supernatant of AD iN cultures by targeted proteomics and single cell RNAseq. Further, fractions of such pro-inflammatory SL iNs can be specifically reduced by applying senolytic drugs to the cultures. Taken together, our data indicate that an increased capacity of AD neurons to enter a SL state could drive or perpetuate widespread neuroinflammation in AD. Taken together, these results establish a model for investigating post-mitotic senescence in human neurons, and suggests that SL neurons could be an exciting target for treating AD which still suffers from scarce therapeutic options.

Keywords: Senescence, Induced Neurons, Alzheimer's

Poster: 775

ENHANCER DELETION OF MICROGLIA-SPECIFIC AD RISK VARIANT BIN1 PRODUCES HYPERPHAGOCYTIC AND INFLAMMATORY PHENOTYPE IN IPSC-DERIVED MICROGLIA

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Microglia, the primary immune cell in brain, are important in development and regulate immune response in neurodegenerative disease. However, their role in the pathogenesis of Alzheimer's disease (AD) remains unknown. AD genetic risk variants have been found in a microglia-specific enhancer regulating the Bridging Integrator 1 (BIN1) locus, suggesting that dysregulation of microglial BIN1 contributes to AD risk. Additionally, BIN1 has 10 isoforms that are differentially expressed in CNS cell types and specific isoforms have been implicated in AD. Yet, it remains unknown what the function of BIN1 in microglia is and how BIN1 AD risk variants alter microglia function. To address this, we leveraged iPSC-derived microglia to determine how loss of the microglia-specific BIN1 enhancer alters microglia function. First, we determined if loss of the BIN1 enhancer reduced BIN1 expression, BIN1 isoform expression, and BIN1 protein levels, using qRT-PCR, RNA-seq, and Western blot. We found that microglial BIN1 expression is regulated by the BIN1 enhancer by showing reduced BIN1 protein and mRNA in BIN1 enhancer knockout (eKO) microglia. Additionally, we found reductions in expression of specific BIN1 isoforms. Next we evaluated microglia morphology and marker expression using a combination of live imaging and immunofluorescence. We determined that BIN1 eKO microglia displayed canonical microglia markers and a ramified morphology similar to control microglia. To then address changes in microglia function due to reduced BIN1 expression, we utilized cell migration, phagocytosis, and lysosomal activity assays. Additionally, we examined inflammatory phenotypes by stimulating with either lipopolysaccharide (LPS) or fibrillar amyloid beta 1-42 (Aβ) and then measuring cytokines. Loss of the microglia-specific BIN1 enhancer resulted in increased lysosomal activity, heightened phagocytosis of Aβ, and enhanced inflammation after both LPS and Aβ. This suggests that loss of the BIN1 enhancer produces phagocytic and inflammatory phenotypes—indicating that loss of BIN1 microglial enhancer harboring the AD risk variant rs6733839 potentially confers AD risk. Thus, by understanding how individual genetic variants contribute to AD we can ultimately develop microglia-specific therapies to target risk before AD onset.

Funding Source: NIH K08 NS109200 CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375

Keywords: Alzheimer's Disease, microglia, enhancer

Poster: 776

FUNCTION OF THE TRINUCLEOTIDE (CGG) REPEATS WITHIN THE FMR1 GENE IN HUMAN NEURONS

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The 5' UTR of the FMR1 gene contains trinucleotide CGG repeats, that, when expanded beyond a certain threshold, can lead to one of several disorders depending on the length of the repeats. Repeats that range from 55 to 200 CGGs lead to either Fragile X Tremor and Ataxia Syndrome (FXTAS) or Fragile X Primary Ovarian Insufficiency (FXPOI), while repeats beyond 200 CGGs lead to silencing of the FMR1 gene and cause Fragile X Syndrome (FXS), a neurodevelopmental disorder that causes 2-5% of all autism cases. Recent research has shown that repeats below 55 CGGs are also correlated with certain health conditions, leading to CGG repeat lengths to be further subdivided into low zone (<24 CGG), normal (24-42 CGG), and gray zone (42-54) categories. To investigate the function of these CGG repeats FMR1 in human neurons, we generated two human embryonic stem cell (hESC) lines lacking the CGG repeats in FMR1 (H1ΔCGG, H13ΔCGG) using genome editing and differentiated these cells into neurons. We found that removal of the CGG repeats does not affect FMR1 expression at the mRNA or protein level in either hESCs, neural progenitors, or early post-mitotic neurons. Interestingly, FMR1 mRNA in neurons lacking CGG repeats exhibit altered localization. Our results demonstrate that CGG repeats in FMR1 may have a function in early neural development, which may have important implications for CRISPR-based gene therapy approaches that aim to remove the expanded CGG repeats from the FMR1 gene.

Funding Source: This study was supported in part by a core grant to the Waisman Center from the National Institute of Child Health and Human Development (U54 HD090256).

Keywords: trinucleotide repeats, human embryonic stem cell-derived neurons, RNA localization

Poster: 777

CHARACTERIZATION OF AN IN VITRO SYNAPTIC PROPAGATION ASSAY USING IPSC-DERIVED NEURONS AND MULTIWELL MICROELECTRODE ARRAY TECHNOLOGY

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Synaptic connections are a fundamental building block of neuronal function, enabling neuronal circuits to process and relay information downstream via action potential propagation. Indeed, many neurological disorders involve impaired connectivity between distinct brain regions. However, traditional in vitro "disease-in-a-dish" neuronal models comprise only a single neuronal circuit, whereas animal models are too costly and complicated to facilitate a screen on compounds or genetic edits that affect synaptic propagation. Previously, we have developed a compartmentalized model of synaptic propagation with rodent cortical neurons. Here, we describe the development and characterization of a simple in vitro assay of synaptic propagation between two distinct neural circuits using iPSC-derived neurons. First, an easy-to-use, two-compartment silicone insert was added to each well of a 6-well microelectrode array (MEA) plate. iPSC-derived neurons, containing a mixture of excitatory and inhibitory neurons, were prepared and seeded into each compartment of the silicone inserts. After two days in vitro, the silicone insert was removed and the networks allowed to mature. The development of functional network activity (e.g., activity, synchrony, oscillations) was monitored every 2-3 days throughout the cell culture period. Activity and synchrony developed first within the networks originally defined by the two-compartment silicone insert, followed by the propagation of network activity from one compartment to the other. Electrical stimulation was used to selectively stimulate one network at a time within each well of the plate while the electrophysiological activity was monitored from the second network. The evoked synaptic propagation was evaluated for each well at baseline, after treatment with either the vehicle control or a cocktail of synaptic blockers (CNQX, APV, Bicuculline), and then following washout. The effect of the synaptic blockade was quantified as the probability and delay of synaptic propagation in response to the stimulus. These results support the continued development and use of in vitro neuronal models and MEA technology for drug toxicity and safety assessment, evaluation of phenotypic disease-in-a dish models, and cell development.

Keywords: Disease-in-a-Dish, Electrophysiology, Synaptic Propagation

Poster: 778

OVEREXPRESSION OF CD47 IS ASSOCIATED WITH BRAIN OVERGROWTH AND 16P11.2 DELETION SYNDROME

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One of the most common genetic linkages associated with neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia, occurs at the 16p11.2 locus. Copy number variants (CNVs) of the 16p gene can manifest in opposing head sizes. 16p11.2 deletion carriers tend to have macrocephaly (or brain enlargement), while those with 16p11.2 duplication frequently have microcephaly. Increases in both gray and white matter volume have been observed in brain imaging studies in 16p11.2 deletion carriers with macrocephaly. Here, we use human induced pluripotent stem cells (hiPSCs) derived from controls and subjects with 16p11.2 deletion and 16p11.2 duplication to understand the underlying mechanisms regulating brain overgrowth. To model both gray and white matter, we differentiated patient-derived iPSCs into neural progenitor cells (NPCs) and oligodendrocyte progenitor cells (OPCs). In both NPCs and OPCs, we show that CD47 (a 'don't eat me' signal) is overexpressed in the 16p11.2 deletion carriers contributing to reduced phagocytosis both in vitro and in vivo. Furthermore, 16p11.2 deletion NPCs and OPCs upregulate cell surface expression of calreticulin (a pro-phagocytic 'eat me' signal) and its binding sites, indicating that these cells should be phagocytosed but fail to be eliminated due to elevations in CD47. Treatment of 16p11.2 deletion NPCs and OPCs with an anti-CD47 antibody to block CD47 restores phagocytosis to control levels. While the CD47 pathway is commonly implicated in cancer progression, we document a novel role for CD47 in psychiatric disorders associated with brain overgrowth.

Keywords: Macrocephaly, 16p11.2 deletion syndrome, CD47 marker, hiPSC, NPC and OPC

Poster: 779

CORRECTION OF PATIENT WLS MUTATION IN INDUCED PLURIPOTENT STEM CELLS USING CRISPR BASE-EDITING

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Wnt signaling is essential for embryonic development and adult tissue homeostasis. The secretion of all 19 Wnt ligands requires a multi-transmembrane protein WNTLESS (WLS), which associates with palmitoylated Wnts in the endoplasmic reticulum and then co-transports to the cell membrane where Wnts are released and WLS is recycled. Our lab has previously reported that homozygous missense mutations in WLS cause a pleiotropic multiorgan condition in humans. Patients exhibited syndromic defects, including microcephaly, facial dysmorphism, renal agenesis, heart and skin defects. Induced pluripotent stem cells (iPSCs) were derived from cultured dermal fibroblast cells of one patient, and were used to generate brain organoids which largely resemble patient microcephaly phenotype. Small-molecule Wnt agonist CHIR99021 was added in culture and successfully increased organoid size. Here, we aim to test if CRISPR base-editing could also be used to correct the patient missense mutation in iPSCs and rescue phenotypes in organoids. Patient sequence-specific sgRNA was designed and cloned into the pgRNA expression vector, which, together with the BE4-Gam vector, was co-transfected into a stable HEK293T cell line with mutant WLS cDNA. In addition to the gRNA and Cas9 nickase, which only cuts the target ssDNA, the CRISPR complex also included an enzyme cytidine deaminase which converts the target nucleotide from a cytosine to a uracil. Results of the

base-editing were analyzed using Sanger sequencing of the PCR products from transfected cells, showing an approximate 20% correction efficiency. Base-editing was further performed in patient iPSCs using nucleofection. Transfected cells were selected with puromycin and efficiency of the base-editing was analyzed. Single cell colonies were selected and genotyped, and the colonies with correct base-editing were amplified and used to generate brain organoids. Altogether, our study shows base-editing could be used to correct patient mutations in iPSCs and rescue phenotypes in cerebral organoids, suggesting a potential application in patient treatment.

Funding Source: CIRM Bridges to Stem Cell Research and Therapy Training Grant, Award #EDUC2-08375 SFARI 51486313 NIH R01NS048453 R01NS09800 UM1HG008900 Broad Institute Center for Mendelian Genomics U54HG006504 Yale Center for Mendelian Disorders

Keywords: Base-editing, WLS, Treatment

Poster: 780

PRECISION MODELS OF ARX-ASSOCIATED GENETIC EPILEPSIES

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Epilepsy is characterized by recurrent seizures due to neuronal hyperactivity. Approximately 75% of epilepsy begins during childhood. ARX is implicated in a wide spectrum of X-linked neurological disorders. Poly-alanine repeat mutations in ARX have been found in patients with mental retardation and epilepsy with no apparent brain developmental abnormalities. Animal models have shown that Arx is critical for cortical interneuron development and migration. In addition, Arx polyalanine expansion (PAE) modifies glutamatergic neurons excitability and morphology. However, brain development differs from mouse to human. Therefore, to elucidate the impact of PAE in ARX gene in human, we have generated “epilepsy-in-a-dish” models from human induced pluripotent stem cells (iPSCs) using cortical and subpallium spheroids (hCS and hSS, respectively). We first detected ARX expression during spheroid differentiation. Then, we generated hCS and hSS from ARX mutated patient and healthy control iPSCs and we analyzed them at different time points by immunostaining. We found a significant increase of progenitor cells and deep cortical neurons at 30 DIV but a decrease of upper layer neurons. In contrast, upper layer neurons were increased at 120 DIV. Similar results were found by single-cell RNA sequencing (scRNA-seq). Moreover, ARX hSS showed interneurons migration defects. These data suggest that ARX affects cell proliferation and neuronal differentiation during human cortical development.

Funding Source: This work was supported by grants from the National Institute of Health and the American Epilepsy Society.

Keywords: ARX gene, Organoids, Interneurons

Poster: 781

HIPPO SIGNALING IN CORTICAL DEVELOPMENT, LAYERING AND DISEASE

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The human cortex is composed of billions of morphologically and functionally distinct neurons. These neurons are produced and organized in a regimental fashion during development. Elucidating the cellular and molecular mechanisms underlying development of the neocortex has been a challenge. Development of the neocortex requires an orchestrated succession of a series of processes, the appropriate generation, migration, and positioning of neurons; the acquisition of layer-specific transcriptional hallmarks, and the establishment of precise axonal projections. The Hippo pathway, a critical regulator of organ size as well as lineage specification in early embryogenesis, is expressed in the developing brain but remains as an unrecognized regulatory module controlling neuronal differentiation and NSC fate commitment. It also remains unexplored in the context of cortical folding and expansion in the developing human brain, hence making it a promising biological question in human cortical development. We have focused on Hippo signaling in the context of the developing human brain, in particular to understand its role in radial and outer-radial glia cells (RG and oRG), the neural progenitor cells present in the germinal zones (GZ) of the neocortex. Using in-silico methods, we are investigating the human specific-downstream targets of Hippo signaling and validating their roles in RG and oRG cells. Using human tissue samples and analyzing the expression of Hippo effectors in developing human brain, we have found Hippo effectors, TEAD1, YAP1, WWTR1, FAT1, FAT4, DCHS1 and CD44 are expressed at RNA and protein level, both in vitro and in vivo, setting the stage for an exploration of their role in development. In parallel, we are elucidating the role of Hippo signaling in cortical folding by making use of a lissencephaly (Miller-Dieker syndrome-MDS) disease model. One central phenotypic characteristic of MDS is microcephaly and the role of Hippo signaling is unexplored in this disease. Employing patient derived MDS iPSCs and cultured cortical organoids, we observe distinct expression patterns for Hippo effectors, suggesting a potential role of Hippo signaling in the disease. In future, gene manipulations will help understand the role that Hippo effectors may play in neuronal differentiation and NSC fate commitment.

Funding Source: Swiss National Science Foundation Early Postdoc Mobility Fellowship

Keywords: Human Cortex, Hippo signaling, Lissencephaly

Poster: 782

GENERATION OF IPSC-DERIVED NEURONS FROM AN ALZHEIMER'S DISEASE PATIENT CARRYING A NOVEL PSEN1 T119I MUTATION

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Alzheimer's disease (AD) is the main cause of dementia in adults. It is estimated that 5% of cases are caused by inherited mutations in genes such as Presenilin-1 (PSEN1). Previously, our group reported a novel heterozygous variant in PSEN1 (c.356C>T; p.T119I) in an Argentine family with early- and late-onset AD. In order to functionally validate this variant, we aimed to generate a human induced pluripotent stem cell (hiPSC) line from dermic fibroblasts (DF) of a male mutation carrier. To this purpose, we first infected patient DF with a STEMCCA lentiviral vector encoding the Yamanaka reprogramming factors (OCT-4, KLF4, SOX2 y c-MYC) and obtained a line termed FFAD1.2. The cells exhibited typical hiPSCs morphological characteristics (formation of compact multicellular colonies with a high nucleus/cytoplasm ratio and distinct colony borders) and high Alkaline Phosphatase (AP) activity. Moreover, we also observed robust expression of different stemness-associated markers (OCT-4, NANOG, SSEA4, TRA1-80, and TRA1-60) analyzed by immunofluorescence and RT-qPCR. Also, using a non-directed method of differentiation (embryoid bodies assay) we demonstrated that this line had the pluripotent potential to be differentiated into cells from the three germinal layers (mesoderm, endoderm and ectoderm) as judged by immunofluorescence analysis of Smooth muscle actin (SMA), Alpha-fetoprotein (AFP) and βIII-tubulin (TUJ1) differentiation markers expression, respectively. Finally, the FFAD1.2c4 presented the same normal karyotype (46, XY) and PSEN1 T119I genotype as the parental DF cells and silenced ectopic expression of Yamanaka gene. Following the generation and characterization of the hiPSC line, we performed a 2D differentiation protocol to generate hiPSC-derived neurons carrying this mutation. After three weeks of differentiation, we observed cells morphologically similar to neurons which expressed high levels of TUJ1 and MAP2, two neuron-specific cytoskeletal protein, analyzed by immunofluorescence. Overall, we successfully obtained a hiPSCs line (FFAD1.2) carrying the AD PSEN1 T119I genotype and achieved to directedly differentiate it to neuronal lineage.

Funding Source: Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI)

Keywords: Alzheimer's disease, Neurodegeneration, point mutation

Poster: 783

SCALABLE DIFFERENTIATION OF HUMAN IPSCS INTO PSEUDO-UNIPOLAR NOCICEPTORS FOR TRANSLATIONAL APPLICATIONS

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Development of new non-addictive pain medications requires advanced strategies to differentiate human induced pluripotent stem cells (iPSCs) into relevant cell types amenable for disease modeling and drug discovery. Here, we devised a highly efficient and scalable protocol that differentiates iPSCs exclusively into nociceptors under chemically defined conditions. By manipulating developmental pathways using small molecules, iPSCs were first converted into SOX10+ neural crest cells followed by differentiation into bona fide pseudo-unipolar BRN3A+ nociceptors. Detailed molecular and cellular characterization and single-cell analysis confirmed that the differentiated nociceptors expressed the neuronal markers, transcription factors, neuropeptides and over 90% of ion channels/receptors expressed in human dorsal root ganglia (DRG). Focusing on pain-relevant receptors and channels expressed by iPSC-derived nociceptors (e.g. P2RX3, TRPV1, NAV1.7, NAV1.8), we demonstrated robust functional activities and differential response to noxious stimuli and specific drugs and suitability for phenotypic screens. Lastly, a robotic cell culture system was used to automate the production of billions of cryopreservable cells for high-throughput drug screening, urgently needed to develop new nociceptor-selective analgesics and help to fight the opioid crisis.

Funding Source: NIH Common Fund, NIH HEAL Initiative

Keywords: Nociceptors, Pain, Opioid crisis

Poster: 784

TRISOMY 21 ESTABLISHES AN OXIDATIVE ENVIRONMENT THAT ACCELERATES NEURAL DIFFERENTIATION OF IPSCS

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Despite an increasing number of studies utilizing Down syndrome (DS) iPSC-derived neurons, characterization of critical intermediate stages – embryoid body (EB), neural rosette, and neural progenitor cell (NPC) – remain overlooked and under-researched. The main aim of the current study was to determine if DS and euploid iPSCs exhibit equivalent neural differentiation potential in vivo. Our laboratory has observed elevated oxidative stress, mitochondrial dysfunction, and altered thiol antioxidant systems in DS iPSCs. As neural differentiation is a redox-dependent process that requires thiol redox homeostasis, a more oxidative environment can lead to DS-specific neuronal phenotypes. The redox state of stem cells modulates the balance between self-renewal and differentiation, with iPSCs having lower respiration and elevated antioxidant enzymes. This establishes a 'reduced' cellular environment that becomes more 'oxidative' with differentiation. Here we

show that DS iPSCs have increased expression of several neural differentiation markers (Pax6, Nestin) and decreased expression of pluripotency (Oct4). We found increased nuclear B-catenin and decreased nucleoredoxin abundance in DS iPSCs. Furthermore, iPSCs produce larger and more EBs than euploid, and show neural rosette morphology two days prior to neural rosette development in euploid EBs. Interestingly, cells present in DS NPC culture express Oct4 and show iPSC morphology. These observations suggest accelerated neural differentiation and reduced progenitor cell development, consistent with elevated OS, mitochondrial dysfunction, and altered antioxidant systems in DS. Due to the lack of previous research on this topic, this experimental data provides evidence that trisomy 21 accelerates neural differentiation due to a more oxidized cellular environment.

Keywords: Down syndrome, Oxidative stress, Embryoid bodies

Poster: 785

SINGLE CELL RNA-SEQUENCING ANALYSIS OF REGIONALLY PATTERNED HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL ORGANOIDS

Chew, Leon H., Mercier, Eloi, Añonuevo, Adam, Hills, Mark, Eaves, Allen C., Louis, Sharon A., Knock, Erin

Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada

Human pluripotent stem cell-derived 3-dimensional neural organoids have been shown to recapitulate the major features and cytoarchitecture of early human brain development. Advancements in the neural organoid culture system from the lab of Sergiu Pașca (Yoon et al., Nat Methods, 2019) was optimized and used as the core technology for the STEMdiff™ Dorsal and Ventral Forebrain Organoid Kits to generate brain-region-specific organoids representing the dorsal and ventral forebrain. As a model of the early brain patterned with developmental cues, these neural organoids contain unique and diverse cell types. Here we sought to investigate the cellular diversity of these organoids using single-cell RNA sequencing, which has become the gold standard in elucidating the cellular composition of complex tissues. We generated dorsal and ventral forebrain organoids using the stem cell line H9 that were subjected to single-cell dissociation on day 50 using an optimized dissociation protocol. We further utilized oligo-tagged antibody cell-hashing to label multiple organoids to explore intra-organoid variability within a culture. Our results show that dorsal forebrain organoids contain a diverse array of cell types including glutamatergic neurons (40 - 60%; LHX2/TBR1/NEUROD6), radial glial cells (20%; TNC/HOPX), and neural progenitors (10%; LHX2/PAX6/TBR2), whereas ventral forebrain organoids contain GABAergic neurons (70%; NKX2.1/GAD1/VGAT/PBX3) and ventral progenitors (25%; NKX2.1/PBX3/ATP1A2). These results indicate the capacity of STEMdiff™ Dorsal and Ventral Forebrain Organoid Kits to generate highly pure forebrain tissue containing cell types observed during development and devoid of contaminating non-neural cell types. Multiplexed samples also showed low intra-organoid variability. Furthermore, comparative analyses revealed that the organoid cell composition and gene expression of key markers are consistent with those observed in organoids from published protocols and were comparable to publicly available data on developing fetal brain tissue. Our results demonstrate that STEMdiff™ Dorsal and Ventral Forebrain Organoid Kits can generate models of the developing telencephalon and enable further study of the complexity of brain development and disorders in vitro.

Funding Source: STEMCELL Technologies

Keywords: Brain Organoids, Single-cell RNA sequencing, Forebrain Development

Poster: 786

CORTICOTROPIN-RELEASING HORMONE (CRH) AFFECTS LINEAGE COMMITMENT OF HUMAN EMBRYONIC STEM CELLS-DERIVED 3D-NEUROSPHERES

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CRH is the major mediator of stress response in mammals. During embryogenesis, CRH/CRH receptors (CRHRs) system is broadly expressed in the developing mouse brain, and exerts anti-apoptotic effects on embryonic NSCs (Koutmani et al., Mol. Psychiatry, 2013). In humans and anthropoid primates, CRH is secreted during pregnancy from the placenta into both maternal and fetal circulations. Since placental hormones are considered to be critical regulators of developmental processes, it is of high interest to investigate the effects of CRH on human embryonic development. To obtain insights into the expression of the CRH system during differentiation of human embryonic stem cells (hESCs) into human neural progenitors (hNPCs), the H9 human embryonic stem cell line was used. hESCs were differentiated into hNPCs and expression of the CRH system was evaluated. While hESCs do not express CRH, they strongly express both CRHRs (CRHR1 and CRHR2). Moreover, human NPCs were maintained in culture as 3D neurospheres and exposed to either CRH or CRHRs antagonists for 40 days. The expression of major developmental, structural and cell-type-specific markers was investigated at weekly intervals to evaluate the differentiation to neurons, astrocytes and oligodendrocytes. Interestingly, exposure of 3D neurospheres to CRH during the differentiation process leads to increased commitment of hNPCs to the neuronal and oligodendrocytic lineage. These observations suggest a direct role of the CRH/CRHRs system in human embryogenesis indicating the putative importance of CRH in human brain development.

Keywords: Corticotropin-Releasing Hormone, placenta, neural stem cells

Poster: 953

DIFFERENTIATION OF SIMIAN INDUCED PLURIPOTENT STEM CELLS INTO BOTH HEPATOCYTES AND CHOLANGIOCYTES

Luce, Eléanor[1], Abed, Soumeya[2], Dubart-Kupperschmitt, Anne[1], Leboulch, Philippe[2], Maouche, Leila[2], Steichen, Clara[1], Weber, Anne[1]

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The use of liver cells differentiated from iPSC cells is an alternative to the use of primary cells which is limited by the lack of donors and the difficulty of their in vitro expansion and cryopreservation. However, before their use in clinical application, the safety and efficacy of this approach have to be established in large animal models, including non-human primates. The aim of this project is to demonstrate that simian iPSCs (siPSCs) can be differentiated into hepatocytes and cholangiocytes as a new cell source for the study of liver development or preclinical therapeutic applications. We reprogrammed the fibroblasts of a macaque and characterized the cells obtained by usual methods. We then differentiated these siPSCs into hepatoblasts (siHBs), the bipotent hepatic progenitors of both hepatocytes and cholangiocytes, by mimicking the main steps of embryonic liver development. The siHBs express specific markers such as HNF4α and the cytokeratin 19 (CK19). We then differentiated these bipotent cells into hepatocytes (siHeps), which expressed specific markers such as HNF4α, albumin, CK8, α1-antitrypsin, SREBP2, and ATP7B. siHeps presented numerous mitochondria, a characteristic of hepatocytes, and displayed epithelial polarity, as shown by the expression of the tight junction protein ZO-1 and the bile salt export pump (BSEP); as well as the excretion at the biliary poles of a fluorescent dye into bile canaliculi. In parallel, adapting our protocol developed for hiPSCs, we differentiated siHBs into cholangiocytes (siChols), expressing SOX9, cytokeratin 7, osteopontin, and the organic anion transporting polypeptide OATP1A2. Finally, in the 3D culture system, siChols self-organized as polarized cysts expressing claudin-7, tetraspanin-15, MDR3, the secretin receptor SCTR, and were able to transport bile acids, visualized with the cholyl-lysyl-fluorescein. In conclusion, we demonstrated that siPSCs enable the production of differentiated liver cells which can be used for the in vitro study of liver development and/or for preclinical models. As we differentiate the hepatocytes and cholangiocytes from the same siPSCs, our results open the way to explore co-culture systems to produce liver tissue that could be used in preclinical studies in a non-human primate model.

Keywords: non-human primate iPSCs, Biliary cells, Liver

13:45 - 14:45 EDT

POSTER SESSION 8

CLINICAL APPLICATIONS

Poster: 802

ANTI-INFLAMMATORY EFFECTS OF ADIPOSE-STEM-CELL CONSORTIA FACTORS AS POTENTIAL TREATMENT FOR ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) INDUCED BY CYTOKINE STORM SYNDROME

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Acute respiratory distress syndrome (ARDS) induced by cytokine storm syndrome (CSS) is caused by unchecked and disproportionate release of pro-inflammatory secretory factors as an immune response to a number of infectious and non-infectious etiologies affecting the lungs. Respiratory infections, especially viral such as H5N1 influenza, SARS-CoV-1, and SARS-CoV-2, can invade lung epithelial cells and alveolar macrophages to produce viral nucleic acid, which stimulates the infected cells to release cytokines and chemokines, activating macrophages, dendritic cells, and others. We have developed a method to create novel consortia of anti-inflammatory and regenerative factors, termed consortia factors (CFx), from adipose derived mesenchymal stem cells (ASC) and tested their effects in vitro. The ASCs were isolated from anonymized biospecimens. Regenerative effects were evaluated with in-vitro wound closure measurements using different concentrations of CFx. The anti-inflammatory effects of CFx were measured by the inhibition of FITC-dextran flow and NF-κB nuclear translocation in endothelial cell (EC) monolayers activated with TNF-α, a pro-inflammatory cytokine. The results of these experiments showed ASC-CFx had regenerative effects similar to those of human fibroblast conditioned media (HF-CM), which was used as a positive control. ASC-CFx were also able to inhibit the inflammatory effects induced by TNF-α on EC monolayers by decreasing the FITC-dextran flow and NF-κB nuclear translocation. These results demonstrate for the first time that ASC derived CFx have strong regenerative and anti-inflammatory effects and may be an effective method to treat ARDS induced by CSS.

Funding Source: Private funds

Keywords: Adipose Stem Cell Secretory Factors, Acute Respiratory Distress Syndrome, Cytokine Storm Syndrome

Poster: 804

CHARACTERIZATION OF A XENOGENEIC FREE, CLOT FREE AND PATHOGEN REDUCED HUMAN PLATELET LYSATE FOR HIGH EFFICIENCY CGMP EXPANSION OF THERAPEUTIC STEM CELLS.

Alonso-Camino, Vanesa[1], Mirsch, William[2]

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Over the past few decades, the number of emerging infectious diseases that spread to people has skyrocketed. The current pandemic caused by Covid-19 outbreak has not only deeply affected human health, but also led to an unprecedented global crisis. Transmission of Covid-19 and other potential emergent pathogens via blood transfusion can be a concern. For that reason, it is likely that blood centers will slowly transition to provide platelets that have gone through a pathogen inactivation process. The effective transfer into the clinic of allogenic cell therapies using MSCs depends predominantly on the development of large scale and cost-effective manufacturing platforms that allow production of functional cells at the scale required to meet clinical demand. Human platelet lysate is one key component in these platforms, as it is used for the efficient cGMP expansion of stem cells. Development of platelet lysates with pathogen reduction processes helps to improve the safety profile for these cell-based therapies. There are several methods approved by the FDA for pathogen inactivation of blood products. We previously validated the use of INTERCEPT™ Blood System platelets to produce pathogen reduced PLTMax® and PLTGold®. However, the global availability of INTERCEPT™ treated platelets is limited at this time. For that reason and due to the increasing concern about transmission of pathogens through the use of blood derived products to expand therapeutic cells, Mill Creek Life Sciences (MCLS) has developed a more sustainable and long-term process to obtain pathogen reduced human platelet lysate (hPL) while maintaining the properties and quality of our products. This process allows us to produce a completely xenogeneic free, clot free and unfractionated human platelet lysate that has been treated with gamma irradiation for pathogen inactivation in a process that maintains unprecedented levels of product quality and efficiency. Here we describe the characterization of gamma irradiated PLTMax® and PLTGold® as well as the studies performed to ensure product sterility.

Keywords: Human platelet lysate, stem cell culture, clinical translation

Poster: 805

IN VITRO SAFETY CLINICAL TRIAL OF THE CARDIAC LIABILITY OF HYDROXYCHLOROQUINE AND AZITHROMYCIN AS COVID19 POLYTHERAPY

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Despite global efforts, there are no effective FDA-approved medicines for the treatment of SARS-CoV-2 infection. Potential therapeutics focus on repurposed drugs, some with cardiac liabilities. Here we report on a chronic preclinical drug screening platform, a cardiac microphysiological system (MPS), to assess cardiotoxicity associated with repurposed hydroxychloroquine (HCQ) and azithromycin (AZM) polytherapy in a mock Phase I safety clinical trial. The MPS contained human heart muscle derived from patient-specific induced pluripotent stem cells. The effect of drug response was measured using outputs that correlate with clinical measurements such as QT interval (action potential duration) and drug-biomarker pairing. Chronic exposure (10 days) of heart muscle to HCQ alone elicited early afterdepolarizations (EADs) and increased QT interval from day 6 onwards. AZM alone elicited an increase in QT interval from day 7 onwards and arrhythmias were observed at days 8 and 10. Monotherapy results closely mimicked clinical trial outcomes. Upon chronic exposure to HCQ and AZM polytherapy, we observed an increase in QT interval on days 4-8. Interestingly, a decrease in arrhythmias and instabilities was observed in polytherapy relative to monotherapy, in concordance with published clinical trials. Furthermore, biomarkers, most of them measurable in patients' serum, were identified for negative effects of single drug or polytherapy on tissue contractile function, morphology, and antioxidant protection. The cardiac MPS can predict clinical arrhythmias associated with QT prolongation and rhythm instabilities. This high content system can help clinicians design their trials, rapidly project cardiac outcomes, and define new monitoring biomarkers to accelerate access of patients to safe COVID-19 therapeutics.

Funding Source: This work was funded in part by the California Institute for Regenerative Medicine DISC2-10090, NIH-NHLBI HL130417, NIH-NIGMS R35GM1195855 and the Jan Fandrianto and Selfia Halim Chair Fund.

Keywords: Cardiac microphysiological system, SARS-CoV-2, polytherapy

Poster: 806

DELIVERY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED SMOOTH MUSCLE PROGENITORS IN COLLAGEN SCAFFOLDS FOR TREATING MURINE ABDOMINAL AORTIC ANEURYSM

Huang, Ngan F.[1], Alcazar, Cynthia[2], Chen, Bertha[3], Hu, Caroline[2], Mulorz, Joscha[4], Tsao, Philip[4], Wen, Yan[3]

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Abdominal aortic aneurysm (AAA) is associated with the loss of resident smooth muscle cells (SMCs) in aortic wall, leading to aneurysmal thinning of the aorta and even fatal aortic rupture. Currently, there are no therapeutic approaches to prevent the progression of AAA. Cell-based approaches to treat AAA are promising, but delivery of cells into the vessel wall is not feasible due to its weakened integrity. The purpose was to evaluate the therapeutic efficacy of delivering iPSC-derived smooth muscle progenitors (iPSC-SMPs) to the adventitia using a collagen scaffold in a murine AAA model. Human iPSC-SMPs or primary human SMCs were seeded at a density of 5x105 cells per scaffold. A murine model of AAA was induced in C57BL/6 mice by local infusion of porcine pancreatic elastase (PPE), followed by daily cyclosporine treatment to prevent immune rejection of human cells. Seven days after PPE induction, collagen scaffolds seeded with either iPSC-SMCs or pSMCs were surgically implanted onto the adventitia of the developing AAA. The survival of the transplanted cells

were tracked by bioluminescence imaging (BLI) and the size of abdominal aortic wall diameters were tracked by ultrasound for 28 days. Primary SMCs and iPSC-SMPs were seeded on porous collagen scaffolds and were shown to maintain smooth muscle phenotypic markers. Following scaffold transplantation on day 7 to the site of AAA, acute BLI demonstrated high cell viability for both primary SMCs and iPSC-SMCs initially. By 28 days after transplantation, however, cell survival gradually declined to 10%. Ultrasound quantification of abdominal aortic diameter revealed that the control group without treatment showed progressive expansion of the vessel diameter. In contrast, the primary SMC-treated animals showed a significantly abrogated expansion of the vessel diameter by 28 days, compared to the control group. Although the iPSC-SMP group did not significantly abrogate vessel expansion, histological analysis showed that iPSC-SMPs promoted more SMC retention in the vessel media, compared to the control group. Our study demonstrates that using collagen scaffold is an effective technique for delivering therapeutic cells in vivo, and that SMC delivery is a promising cell therapy for treatment of AAA.

Funding Source: California Institue of Regenerative Medicine; American Heart Association

Keywords: cardiovascular disease, smooth muscle cell, tissue engineering

Poster: 807

Assessing Contractility of 3D iPSC-derived Engineered Muscle Tissues for Safety and Drug Discovery Application Using a High-throughput and Novel Label-free Method

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Stem cell models hold great promise for improving the predictive power of preclinical in vitro assays for new therapies and drug discovery. Complex 3D platforms, such as Engineered Muscle Tissues (EMTs) fabricated from primary or iPSC-derived cells can directly measure tissue contractility, which is challenging in traditional 2D platforms where cells are rigidly attached to a surface. These contractility measurements can be used to directly assess the potential toxicity of therapeutics for both acute proarrhythmic and chronic structurally toxic compounds. However, the fabrication of EMTs traditionally demands extensive bioengineering expertise and measuring contractility has often involved laborious, serial, and low-throughput optical measurements. Here, we report on the design, fabrication, and validation of a novel EMT platform that uses 1) facile and scalable bioengineering approaches to generate tissues from a variety of cell sources, and 2) a label-free parallel measurement technique. Our tissue casting approach improves success rate to >95% (n > 100) and produces consistently-sized constructs with a standard deviation of +/- 9% across 6 experiments. The substrate features an embedded magnet; as tissues contract, the magnet's displacement is quantitatively detected in a highly-parallel manner using specialized sensors. We detected 24 contractions simultaneously with a rate of measurement 100Hz, which is suitable for measuring various aspects of contractility such as upstroke velocity and decay time. We demonstrated that the signal voltage changes are linear with respect to EMT contraction. Here, we will focus on Engineered Heart Tissues (EHTs) and will present data showing acute inotropic and chronotropic effects of drugs measured minutes after EHT dosing. Further, we will demonstrate the chronic effects of structural cardiotoxicants like doxorubicin on EHTs. Control and dosed tissues showed no differences at acute (30 min) or daily timepoints to Day 2 post exposure. The highest dosed (1 μM) tissue began to slow at Day 4 ($p < 0.01$) and Day 5 ($p < 0.0001$) until complete cessation of beating by Day 6. In summary, we have designed a novel system that can leverage the complexity of 3D cellular models, but still support the needed high throughput for contemporary drug discovery.

Keywords: 3D Engineered Muscle Tissues, Safety and Drug Discovery, High-throughput Contractility Measurements

Poster: 808

CRITICAL QUALITY ATTRIBUTES IN PLURIPOTENT STEM CELLS BANKING: THE ROLE OF CELL VIABILITY

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Quality control (QC) testing is required during cell banking and post-banking stages, to ensure quality and safety of cell banks. The UK Stem Cell Bank (UKSCB) has developed a QC testing strategy, which is comprised of several tests and associated acceptance criteria that identify human pluripotent stem cell (PSC) lines suitable as starting materials for advanced therapy medicinal products (ATMPs). Cell viability assessment is one of UKSCB's mandatory QC tests, used to provide an indication of post-thaw recovery of cells following cryopreservation and of cell quality during subculture stages. Using 21 high-quality datasets collected during post-banking stage of human embryonic stem cell (hESC) lines under the EU Tissues and Cells Directive, we evaluated the impact of cell viability acceptance criteria in providing a high degree of quality assurance for clinical applications suitability. The selection of 21 feeder-dependent and feeder-free hESC line banks was based on a set of 8 critical quality attributes (CQAs). Examination of cell viability percentages and split ratios (as a measure of expansion ability) of cell banks at thawing and two subsequent subculture stages has shown that a stringent acceptance criteria for cell viability (≥70% viable cells) is not, in isolation, representative of cell banks' ability to recover and expand after cryopreservation. Our data strongly indicate that cell viability acceptance criteria can be set to lower values to identify high-quality banks, if it is used in combination with other criteria, namely cell morphology and expansion ability. As a result, the UKSCB has revised its viability acceptance criteria to the lowest viability percentage value observed across the 21 hESC line banks analysed. Ongoing studies are focused on the continuous enrichment of data for viability characteristics of clinical hESC lines and will be expanded to the rest of our CQAs to ensure consistent and reliable delivery of high-quality cellular products.

Keywords: embryonic stem cells, advanced therapy medicinal products (ATMPS), quality control testing

Poster: 809

MAINTENANCE AND EXPANSION OF HIGH QUALITY HUMAN PLURIPOTENT STEM CELLS (HPSC) USING A NOVEL ANIMAL ORIGIN-FREE AND STABILIZED HPSC MAINTENANCE MEDIUM

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As the number of clinical trials and approved therapies in the field of regenerative medicine increases it is important to ensure that hPSC culture media are not only compliant with current required manufacturing and quality control processes but also do not create bottlenecks to final regulatory approval. To simplify traceability and viral safety concerns, we have developed an animal origin-free (AOF) hPSC maintenance medium, TeSR™-AOF, manufactured under cGMP using animal-free raw materials with traceability to the secondary level of manufacturing. Key media components, including native FGF2 in TeSR™-AOF, are stabilized over 72 hours at 37°C to support reduced feeding schedules with every other day or weekend-free feeding. Additionally, buffering capacity was optimized to stabilize pH under these restricted feeding schedules. To improve expansion across hPSC lines, TeSR™-AOF was also optimized to improve plating efficiency compared to low-protein media. Typically, plating efficiency 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. We investigated key cell quality attributes of hPSCs cultured for ≥10 passages in TeSR™-AOF with reduced feeding schedules. hPSC marker expression was assessed by flow cytometry at passages 5 and 10 and hPSCs cultured in TeSR™-AOF maintained an average of 97.7 ± 3.01% OCT4 and 93.2 ± 5.27% TRA-1-60 (n=4). Furthermore, hPSCs maintained in TeSR™-AOF were capable of directed differentiation to all three germ layers using the STEMdiff™ Trilineage Kit (n=2) and were karyotypically normal by G-banding using the hPSC Genetic Analysis Kit (>10 passages, n=4). In summary, TeSR™-AOF improves hPSC attachment efficiency and consistency, whilst enabling versatile workflows and preserving high quality hPSCs in long term culture.

Keywords: Pluripotent stem cells, Animal origin free, cGMP

Poster: 810

LARGE-SCALE EXPANSION AND CRYOPRESERVATION OF HUMAN IPSC-BASED HEPATIC ORGANOIDS

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Organoids are three dimensional, self-organizing cell aggregates that resemble the structure and function of organs. Hepatic organoids are considered of special interest in biomedical research for disease modelling or drug screening purposes. Since the source and expansion capabilities of primary human hepatocytes (PHH) are still limited, human induced pluripotent stem cells (iPSC) are considered a feasible replacement of PHH due to their capability for large-scale expansion to meet the increasing demand in biomedical research. Here, we present the generation of hepatic organoids from iPSC via definitive endoderm and hepatic progenitor cells. The optimized differentiation process has been translated to scalable suspension-based and impeller-free bioreactors to meet the increasing demand of this diagnostically relevant cell system. In pilot studies, the technical settings have been optimized by adjusting the initial seeding density, rotation speed, inoculation time, and medium viscosity in order to produce homogeneous hepatic organoids and to maximize the biomass yield. The medium composition has furthermore been optimized to enhance the maturity and functionality of the resulting organoids, which has been validated by the assessment of different hepatocyte markers including HNF4A, AFP, ALB, A1AT and CYP3A4 by gene expression and immunohistochemistry analyses. The complete resulting differentiation process from iPSC's to mature organoids takes around one month. In order to speed up the production process by starting from intermediate products and to store mature hepatic organoids, cryopreservation approaches for the controlled freezing of 3D cell systems are being analysed at two time points along the differentiation regime regarding cell recovery, gene expression, and immunohistochemistry analyses. Collectively, these studies will allow the production of large batches of hepatic organoids via expansion in bioreactors for biomedical studies.

Funding Source: This work has been funded by the Innovative Medicines Initiative JU, grant agreement 821362, composed of financial contribution from the European Union and EFPIA companies' in kind contribution.

Keywords: Hepatic spheroids, Cryopreservation, Bioreactor

Poster: 811

GENERATING HUMAN PLURIPOTENT STEM CELL-DERIVED LIVER CELLS

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Each year, more than 1 million people worldwide die of chronic liver failure. At present, whole-organ liver transplantation is the only effective long-term treatment for liver failure, but this treatment option is vastly limited by a severe shortage of donor organs. Thus, there is an imperative need for new approaches to treat liver failure. Human pluripotent stem cells (hPSCs) have the capacity to self-renew and have the potential to differentiate into many diverse cell-types. Here, we developed a strategy to efficiently generate large quantities of highly-pure hepatocyte-like cells from human embryonic stem cells (hESCs). These hESC-derived hepatocyte-like cells expressed high levels of human ALBUMUN and other hepatocyte markers. We also demonstrated that these cells can engraft robustly in the injured mouse liver. This strategic approach opened doors to new possibilities in meeting a critically unmet clinical need, that is to obtain large numbers of hepatocytes to treat patients with liver failure.

Funding Source: California Institute for Regenerative Medicine (CIRM), The Thomas and Stacey Siebel Foundation; Stanford Maternal and Child Health Research Institute (MCHRI)

Keywords: hepatocytes, human embryonic stem cells, cellular transplantation

Poster: 812

A CRISPR- AND IPSC-MEDIATED THERAPY FOR DYSTROPHIC EPIDERMOLYSIS BULLOSA

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Dystrophic Epidermolysis Bullosa (DEB) is caused by a plethora of recessive or dominant mutations of the COL7A1 gene. In normal skin, the product of the COL7A1 gene functions like a 'molecular glue' that keeps skin layers attached to each other. DEB patients lack this 'molecular glue' and suffer from chronic erosion of the skin, leading to blistering, open wounds, infection, and ultimately, a high risk to develop squamous cell carcinoma. Current treatment is limited to wound care but with the advent of stem cell technology and CRISPR-mediated genome engineering curative therapies are within reach. We have successfully developed a new therapeutic strategy currently approaching the pre-IND phase of regulatory approval by the FDA. Our approach is based on patient-derived induced pluripotent stem cells (iPSCs), genetic correction of the pathogenic COL7A1 mutation, and subsequent differentiation into autologous keratinocyte sheets that can be grafted for definitive treatment. Importantly, iPSC derivation from primary patient tissue and CRISPR-mediated correction of pathogenic COL7A1 mutations has been integrated into a 1-step protocol that allows isolation of COL7A1-edited iPSCs within less than 1 month, making clinical translation feasible and significantly reducing mutational load caused by prolonged ex vivo culturing of cells. Our approach permits selection of genome-engineered iPSCs that are free of cancer-causing mutations, which exist in donor skin cells, is fully compatible with GMP-regulations, and leaves the genome unperturbed (i.e. maintenance of a normal karyotype, no integration of selection cassettes etc.). The employed CRISPR-Cas9 system is highly specific and mutations were not detected at any of the predicted off-targets. Keratinocyte sheets derived from these engineered patient-iPSCs express functional COL7A1 and successfully graft onto devitalized human dermis and mouse models

Funding Source: The California Institute for Regenerative Medicine and DEBRA (CIRM) The Dystrophic Epidermolysis bullosa research Association (DEBRA)

Keywords: CRISPR & iPSC Mediated therapy, Dystrophic Epidermolysis Bullosa, COL7A1 gene

Poster: 813

EFFICIENT CULTURE OF TISSUE-DERIVED HUMAN HEPATIC ORGANOIDS USING THE HEPATICULT ORGANOID KIT

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Liver organoids represent a physiologically relevant alternative to conventional two-dimensional cell culture for disease modeling, toxicity screening, and the study of liver biology. The HepatiCult Organoid Kit (Human) is a novel cell culture system that supports a complete liver organoid culture workflow, including organoid establishment, expansion, and differentiation for downstream applications. Ductal material from liver biopsy-derived normal tissue was cultured in HepatiCult Organoid Initiation Medium to yield organoids within 2 weeks (n = 14). Established organoids were passaged every 4 - 10 days in HepatiCult Organoid Growth Medium; these organoids expressed proliferation (KI67), ductal (SOX9), and hepatic (HNF4A) markers (n = 6). Upon further differentiation in HepatiCult Organoid Differentiation Medium, organoids exhibited increased mature hepatocyte marker (ALB, CYP3A4) expression and decreased stem cell and ductal marker (LGR5, CK19) expression, as assessed by qPCR (n = 6), as well as a 56 ± 8-fold increase in albumin secretion, 10 ± 4-fold increase in baseline CYP3A4 activity, 25 ± 11-fold increase in bile acid production, and 6.9 ± 1.9-fold increase in urea production (n = 3, mean ± SEM), relative to undifferentiated organoids. Mature organoids also exhibit higher sensitivity to ketoconazole-induced hepatotoxicity (IC50 = 13.8 µM; 95% CI = 9.86 - 19.3 µM) as compared to the HepG2 hepatoma cell line (published IC50 = 112 µM) (n = 2 experiments, 3 technical replicates per experiment). These results demonstrate that the HepatiCult Organoid Kit is a robust tool for the culture of tissue-derived hepatic organoids.

Keywords: Liver, Organoid, Hepatocyte

Poster: 814

DESIGNING PERSUASIVE HEALTH EDUCATION FOR PATIENTS SEEKING UNPROVEN STEM CELL INTERVENTIONS

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A looming ethical issue in regenerative medicine surrounds the unproven stem cell and regenerative medicine intervention (SCRI) market. This for-profit industry is built on ambiguous science with the US having the largest market. Patients having undergone unproven SCRIs have experienced significant physical, economic, social and emotional harms. The market thrives primarily through promotion of hyperbolic claims of benefits, undermining risks, and spreading disinformation. Most

patients see benefits, praise providers for giving hope, and recommend SCRIs to other patients. To curtail this industry, the scientific community has focused efforts to enhance regulations and enforcement which has been met with little success as the market continues to grow. Little scientific attention has been paid to effectively inform and educate patients. Although several organizations, including ISSCR, have begun developing patient education resources, these predominantly text-based materials rely on didactic learning of scientific facts that require high health literacy skills and laborious rational weighing of risks and benefits. These approaches are not tailored to patient needs, lack persuasive appeal, and discount theories of health behavior change and healthcare decision-making. In this presentation, we will describe existing educational approaches and present a theoretical framework based on theories of health behavior change and communication to outline an approach to design effective health education that has greater persuasive appeal and can combat misinformation about unproven SCRIs. We will provide a moral justification for using persuasive communication approaches for patients and delineate variables known to influence health behavior including source credibility, self-efficacy, trust, and social influence. We will describe measurement of key constructs to evaluate communication impact and inform communication strategies. The design of persuasive communication is of paramount importance to the scientific and medical communities and should be adopted in the context of patient-physician consultation and public health campaigns designed to inform out-of-option patients considering dubious SCRIs.

Keywords: unproven stem cell interventions, persuasive health communication & education, ethics and autonomy

Poster: 815

ESTABLISHING CHEMOKINE GRADIENTS ACROSS THE MOUSE RETINA ENHANCES DONOR RETINAL GANGLION CELL INTEGRATION

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Glaucoma and other optic neuropathies lead to permanent loss of retinal ganglion cells (RGCs) in the neural retina of the eye. Cell transplantation has been proposed to restore RGCs; however, current studies show limited structural integration into the neural retina following transplantation. Here, we use known chemokines to improve RGC integration and establish either stromal cell-derived factor-1 (SDF1) or netrin-1 gradients across the retina before donor RGC delivery. For all the studies below, RGCs are differentiated from Thy1-GFP mouse iPSC in 3D retinal organoid cultures and isolated by magnetic microbeads against CD90.2 at day 21 of differentiation. Thy1-GFP+ RGC suspension (2 µL, 5 x 106 cells/mL) is delivered intravitreally (IVT) immediately after subretinal injection of SDF1 (10ng) or netrin-1 (100ng). In a "reverse" set of experiments, RGCs are injected subretinally, and SDF1 or netrin-1 is injected intravitreally. Two weeks after transplantation, retinas are collected and stained for GFP and RBPMS to assess donor RGC integration and distribution across and within the retina with respect to host ganglion cell layer (GCL), visualized by staining for RBPMS+ cells. We also analyze the neurite length and the morphology of donor RGCs to see their capacity to extend their processes towards the inner plexiform layer and optic nerve head. With histological analysis, we have not observed any major side effects of cells or chemokine administration on retinal morphology. Thy1-GFP+ donor RGCs integrate into host retinas with and without artificial SDF1 gradient and have the same morphology in both groups, netrin-1 gradients increase the formation of donor RGC processes within the host retina (X^2 (1, N = 152) = 7.6325, $p < .05$). The SDF1 gradient increased the retinal coverage by donor cells from 54 ± 8% to 76 ± 4% ($p < 0.05$), proposing a benefit of CXCR4-SDF1 interactions for enhanced donor RGC migration. These findings are coherent with the expression of receptors for SDF1 and Netrin1 (CXCR4, CXCR7, DCC, UNC-5C, and DSACM) on donor RGCs. Altogether, our studies confirm that it is possible to control donor cell fate behavior by modulating tissue microenvironment and SDF1 and netrin-1 gradients across the host retina are effective strategies to improve the distribution and structural integration of donor RGCs.

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Keywords: Retinal Organoid, Cell Replacement, Glaucoma

Poster: 816

SOLUBLE CX3CL1-EXPRESSING HUMAN RETINAL PIGMENTED EPITHELIUM CELLS DELAY PHOTORECEPTOR DEGENERATION IN A MOUSE MODEL OF RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is an inherited retinal degenerative disorder that causes severe vision loss or blindness, affecting nearly 1 in 3500 people. Since any single mutation in over 70 identified genes can contribute to this devastating disease, developing multiple gene replacement therapies may prove infeasible. While RP exhibits genetic heterogeneity, it results in a common clinical progression of primary rod and secondary cone photoreceptor degeneration; consistent with this, various groups have demonstrated that microglial activation represents a non-cell autonomous mechanism of photoreceptor degeneration, which may allow for the development of therapeutics that are beneficial to all patients (independent of the mutation they carry). Soluble CX3CL1 (sCX3CL1) has been shown to delay photoreceptor degeneration by inhibiting microglial activation and to promote the long-term preservation of photoreceptors in mouse models of RP. To overcome the risks and limitations of gene therapy and frequent intravitreal injections, we propose the solution of delivering human pluripotent stem cell-derived retinal pigmented epithelium cells (RPE) into the subretinal space as a factory for sCX3CL1. These therapeutic cells will be built on the two platform technologies of our lab that address issues of cell therapy safety (Safe Cell) and allogeneic rejection (induced allogeneic cell tolerance). Here, we show that our genetically engineered RPE express markers that confirm the RPE cell fate, as indicated by flow cytometry and immunocytochemistry, and express sCX3CL1 both in vitro and in vivo. Furthermore, optical coherence tomography and immunohistochemical analyses suggest that sCX3CL1-expressing RPE not only integrate

into the subretinal space, but may also delay photoreceptor degeneration by inhibiting microglial activation. Thus, we hypothesize that the cell-mediated sCX3CL1 therapy can delay photoreceptor degeneration by inhibiting microglial activation in a controlled and long-term manner. Overall, this approach has the potential to delay the progression of RP, and its success will provide insight into developing treatments for other neurodegenerative diseases.

Funding Source: Canadian Institutes of Health Research Foundation Grant and Fighting Blindness Canada

Keywords: Vision Loss, Cell Therapy, Retinal Degeneration

Poster: 817

QUINACRINE-CASIN COMBINATION OVERCOMES CHEMORESISTANCE IN HUMAN ACUTE LYMPHOID LEUKEMIA

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Chemoresistance posts a major hurdle for treatment of acute leukemia. There is increasing evidence that prolonged and intensive chemotherapy often fails to eradicate leukemic stem cells, which are protected by the bone marrow niche and can induce relapse. Thus, new therapeutic approaches to overcome chemoresistance are urgently needed. By conducting an ex vivo small molecule screen, here we have identified Quinacrine (QC) as a sensitizer for Cytarabine (AraC) in treating acute lymphoblastic leukemia (ALL). We show that QC enhances AraC-mediated killing of ALL cells, and subsequently abrogates AraC resistance both in vitro and in an ALL xenograft model. However, while combo AraC+QC treatment delays ALL relapse in primary transplanted recipients, the combination exhibits limited efficacy in secondary transplanted recipients, consistent with the survival of niche-protected leukemia stem cells (LSCs). Introduction of a newly identified Cdc42 Activity Specific Inhibitor, CASIN, enhances the eradication of ALL leukemia stem cells by AraC+QC and delays ALL relapse in both primary and secondary transplanted recipients without affecting normal long-term human hematopoiesis. Together, our findings identify a small-molecule regimen that sensitizes AraC-mediated leukemia eradication and provide a novel potential therapeutic approach for better ALL treatment.

Funding Source: This work is supported by an NIH/National Heart, Lung, and Blood Institute (NHLBI) grant (R01HL151390 to W.D.).

Keywords: Acute lymphoid leukemia, Chemo-resistance, Hematopoietic stem progenitor cells

Poster: 818

IMMUNE TOLERANCE ENVIRONMENT INDUCTION FOR TRANSPLANTATION

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The ability to generate all kinds of pancreatic endocrine cell types makes it a huge promise for cell replacement therapy for diabetes. Nowadays, the two main kinds of islet cells—insulin-producing beta cells and glucagon-producing alpha cells in vitro differentiation are both successes in the lab. After getting a cell source, the major obstacle for cell therapy is the rejection of foreign cells by the recipient's immune system. Many efforts have been put into how to prevent allo-rejection. Here we use the naturally exist immune privilege organ to build an immune tolerance microenvironment to protect the allo-transplanted graft. Immune privilege organs are categorized as their properties of remarkable immune privilege and effective local innate immunity. Mammal testis is one of the well-known immune privilege organs. To protect immunogenic germ cells from systemic immune attack, testicular immune privilege is maintained through the coordination of systemic immune tolerance, the local physical structure, blood-testis-barrier(BTB), and active local immunosuppression. Here we report that mouse testis could serve as protection of transplanted human stem cell-derived islet (SC-islet) cells. The cells are protected both inside and outside of the BTB, and they showed a proper function of accurately regulating blood sugar levels in the interstitium of the testis. In in vitro co-culture with mononuclear cells (MNCs) assay, we observed suppressed T cell activation and proliferation in presence of Testis tissue or Sertoli cells in the testis. We also noticed a special group of T cells that has both CD4 and CD8 expression exist in both in vitro assay and in vivo transplantation. We show that this population of T cells resembles T regulatory (T reg) cells' feature and act as a protective role of SC-islet. Soluble signals secreted by testis cells especially Sertoli cells could induce this population and TGF-β plays an essential role in the induction of this CD4/CD8 double-positive population. This population of T cells could also be protective outside the testis structure. Our study provides a solution for immune protection of stem cell-derived organ transplantation from a new angle.

Keywords: immune protection, transplantation, regulatory T cells

Poster: 819

A UNIVERSAL CYTOTOXICITY ASSAY FOR POTENCY TESTING OF T CELL THERAPIES

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Cellular therapy products are heterogeneous mixtures with complex mechanisms of action. In-depth product characterization is necessary in order to determine product quality attributes. These critical quality attributes are assigned acceptance ranges to ensure lot-to-lot consistency while ensuring safety and efficacy. Identity, purity, and potency of biological products commonly rely on cell surface markers analysis using flow cytometry and cell-based assays or surrogate measurement of secreted molecules to assess functionality. Current approved T-cell based therapies utilize these assays, but there is an increasing need for better performing, faster and more predictive analytical solutions. We had earlier reported the development of the PureQuant assay, a cell type-specific, epigenetic qPCR-based method that is sensitive, consistent, and standardized with low sample requirement ideal for identity and purity testing. Here, we report the design and development of a universal target cell line for cell-based functional assay. CAR-T cells recognize a specific surface antigen to identify and specifically kill the target

cell. Preclinical models should therefore have both control and target cells to generate data supporting antigen specific lysis. K562 cells have been extensively used as a cell target due to lack of CAR-T cell antigens and HLA protein complexes thereby reducing assay background. K562 are commonly transduced with lentiviruses to express the target antigen of interest. Because integration of target genes is random and there is no way to control the context of expression, development of robust cell-based cytotoxicity assays using LV mediated gene delivery is very challenging. To overcome this, we have designed and developed a K562 line using Jump-In technology by placing a single copy of the R4 integrase site which upon retargeting will drive expression of desired antigen(s), along with GFP and Luciferase. The co expression of the desired antigen, GFP and Luciferase enables flow cytometry or plate-based cytotoxicity assays to measure the antigen specific lysis of target cells by CAR-T cells. Using CD19 as the antigen, we demonstrate utility of this platform K562 cell line in cytotoxicity assays for the functional analysis of CD19 CAR-T cells.

Keywords: CAR-T, cytotoxicity, functional assay

Poster: 820

OPTIMIZATION OF HUMAN SMOOTH MUSCLE CELL DIFFERENTIATION MEDIA FOR CLINICAL MANUFACTURING

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Human iPSC-derived therapies are promising options for degenerative condition, but their large-scale production is challenging due to the availability of well-defined, cost-effective and clinical-grade reagents. Our previous studies have shown in-vivo efficacy of human iPSC-derived smooth muscle progenitor cells (pSMCs) cultured in Gibco Smooth Muscle Growth Supplement (SMGS) medium for treatment of stress urinary incontinence. However, GMP-grade Gibco SMGS is not available, thus our study goal is to evaluate GMP-compatible SMC differentiation media. iPSCs were derived from human dermal fibroblasts and differentiated into CD34+ precursor cells as previously described. These cells were then further differentiated into pSMCs in these media groups: Gibco SMGS, DMEM/F12 HEPES & 5% fetal bovine serum (FBS), Cell Biologics & 5% FBS, Cell Biologics & 5% human serum, and M231 & 5% FBS. pSMC at passage 4 (P4) and terminal smooth muscle cells (tSMCs) at P5 were evaluated for pluripotency and SMC markers by q-PCR (OCT4, SOX2, Nanog, LIN28, SM22, αSMA, SMT, CNN1, MCH11, Elastin) and flow cytometry (CD30, CD31, CD34, αSMA, TRA-1-60, TRA-1-81, SMT). Contractility of tSMCs were accessed by carbachol assay. DMEM/F12 HEPES & 5% FBS is the most cost-effective and chemically simple of the media groups. pSMCs cultured in this medium displayed high levels of smooth muscle markers (SM22, CNN1, HCM11, and elastin) relative to other groups, and similar pluripotency as bladder smooth muscle cells harvested from a female 50-year-old patient. Contractility of tSMCs cultured in DMEM/F12 HEPES & 5% FBS is higher than than those cultured in Gibco SMGS. These data suggest that DMEM/F12 HEPES & 5% FBS may be a viable differentiation medium for clinical manufacturing of iPSC-derived smooth muscle therapies.

Funding Source: This project was funded by the California Institute for Regenerative Medicine (CIRM) TRAN1-10958, PI B.Chen.

Keywords: Smooth Muscle Differentiations, Smooth Muscle Differentiation Media, iPSC-Derived Progenitor Smooth Muscle Cells

Poster: 822

STEM CELL THERAPY FOR URINARY INCONTINENCE: SAFETY OF PERIURETHRAL CLINICAL INJECTION SITE IN MICE

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Human iPSC-derived smooth muscle progenitor cells (pSMC) display potential for treatment of stress urinary incontinence (SUI). However, due to the tumorigenic potential of stem cells, safety concerns present a challenge for its translation to the clinic. Teratoma formation and potency is dependent on the engraftment site. Given numerous innate and adaptive immunities within different non-lymphoid tissue environments, cellular viability and localization after transplantation may vary by injection site. In spite of numerous studies on stem cell therapy for SUI, safety of the periurethral clinical injection site for SUI has not been examined. Thus, we sought to evaluate teratoma formation and iPSC distant migration after periurethral injection of iPSC in the murine model. iPSC were reprogrammed from patient-derived dermal fibroblasts using a mRNA/miRNA protocol with confirmation of expression of pluripotency markers (Nanog, Oct-4, Sox-2, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81). NSG immuno-deficient mice were injected with different doses of iPSC: 1) 5 x 106 iPSC – leg intramuscular (n = 5), 2) 5 x 106 iPSC – periurethral (n = 8), 3) 4 x 106 iPSC – periurethral (n = 8), and 4) intact control (n = 3). Mice were monitored and organs harvested post-injection at days 1, 5, 30, and two months. Immunohistochemistry (IHC) for human nuclei and hematoxylin eosin (HE) stains were performed on all tissues and extracted tumors. DNA from the mouse brain, liver, kidneys, spleen, bladder, vagina, and urethra was extracted and the number of human cells was quantified using Alu-seq qPCR. IHC confirmed successful periurethral injection with co-location of human cells at day 1 post-injection for the 4 x 106 and 5 x 106 iPSC groups. Histological analysis on the tumors revealed tissue types from all three germ layers, validating positive teratoma formation. Alu seq qPCR detected a quantifiable number of human cells in various organs. These data confirm positive teratoma formation at periurethral injection site and migration of iPSCs to distant organs post periurethral injection. Further studies are needed to elucidate safe threshold levels for iPSC contamination in differentiated cell product for clinical applications.

Funding Source: Acknowledgements and Funding: This project was funded by the California Institute for Regenerative Medicine (CIRM) TRAN1-10958, PI B. Chen

Keywords: Safety, iPSC-derived smooth muscle progenitor cells, Stress urinary incontinence

Poster: 823

R2U-TOX-ASSAY PROJECT: BRINGING HUMAN DIVERSITY IN A 96 WELL PLATE FOR IMPROVED DRUG DEVELOPMENT

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Human cell-based assays are becoming an indispensable tool for drug research, contributing significantly to safety and success in drug development. However, the current standard is animal cell models with limited translatability to the human system, leading to increased risks for patients in clinical studies due to toxic side effects and even to market withdrawals of final products. The project R2U-Tox-Assay, applies human induced pluripotent stem cells (hiPSCs) to generate significant cell systems and predict drug-induced toxicity at an early stage in development. Cardiac and neural cell types will be in focus as cardiovascular and neurological side effects are responsible for the exclusion of a large proportion of drug candidates (> 24 %). Even though all parts in the production chain from hiPSCs to specific cell types applicable in drug screenings are published, the workload and intrinsic knowledge to generate assay plates are very high for end users and there is currently no option for stock keeping of a produced batch. In contrary, such assay plates have to be produced on demand in time-intense differentiation processes and show a limited shelf life. Therefore, there is an urgent need for the supply of significant and ready-to-use assay plates containing specific human cell models to assess drug-induced toxicities. R2U-Tox-Assay will establish such a centralized service comprising the production of specialized cell types using scalable platform technologies as well as their stock keeping in a ready-to-use ('R2U'), adherent format. For the latter, an innovative cryopreservation strategy is realized, enabling storage and shipping of adherent cells in a 96 well plate format, ready to be thawed and immediately applied by end users. In addition, the generation of target specific reporter cell lines via CRISPR-Cas9 technology will fasten targeted readouts and increase R2U-Tox-Assays' benefit for pharmaceutical research.

Funding Source: This work has been funded by EIT Health, grant agreement ID 20366.

Keywords: Drug screening, Neural and cardiac cells, Cryopreservation

Poster: 824

DERIVATION OF OTIC NEURONAL PROGENITORS FROM HYPOIMMUNOGENIC IPSCS: A NOVEL APPROACH FOR STEM-CELL REPLACEMENT THERAPY IN THE INNER EAR

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Although stem cell replacement therapy holds potential for sensorineural hearing loss (SNHL) treatment, transplanted cells' limited engraftment poses a challenge for its translation into clinical practice. The reasons for limited engraftment include clearance by a resident immune cell population in the cochlea. To circumvent this issue, we propose to generate otic neural progenitors (ONP) derived from engineered induced pluripotent stem cells (iPSC) that evade immune detection and response. We hypothesize that this approach will lead to prolonged residence time in the cochlea, improving cell engraftment and tissue restoration. The hypo-immunogenic iPSC (hi-iPSC) line used in this study was generated by gene editing technology-mediated deletion of HLA genes and by overexpression of the immune cloaking genes HLA-G and PD-L1. Differentiation of hi-iPSCs to ONP was performed following our already established protocol. Flow cytometry analysis, immunocytochemistry and quantitative PCR were performed to assess hi-iPSCs and ONPs phenotype and to quantify expression of HLA-G and PD-L1. All experiments were performed using wild-type iPSCs (wt-iPSC) as control. Our data indicate that genetic modifications in hi-iPSCs do not impair their viability, colony formation capacity, expression of pluripotency markers and differentiation towards ONPs. Hi-iPSC presented increased surface expression of HLA-G and PD-L1 (compared to wt-iPSCs), which, however, gradually decreased throughout differentiation. The impact of the reduced expression of HLA-G and PD-L1 in the immunogenicity of the derived ONP needs to be investigated before their use in preclinical SNHL models.

Keywords: Stem cell engineering, Hypoimmunogenic derivatives, Inner ear regeneration

Poster: 826

BIOCHEMICAL VALIDATION OF A COMPUTATIONAL REPRESENTATION OF THE DIFFUSION PROFILE OF BRAIN DERIVED NEUROTROPHIC FACTOR ON A MICROFLUIDIC CULTURE PLATFORM

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Sensorineural hearing loss (SNHL) is the most common permanent hearing impairment. One potential treatment strategy for SNHL is to regenerate the neural network in the inner ear using human pluripotent stem cells (hPSCs). This

process is dependent on neurite outgrowth, which is directed by brain-derived neurotrophic factor (BDNF). We have previously used the POlyhedrin Delivery System (PODS, Cell Guidance Systems, Cambridge, UK) to promote sustained release of BDNF in the inner ear of mice. However, substantial in vivo neurite growth requires that the concentration of BDNF remains sufficiently high for several weeks, and the diffusion kinetics of BDNF in the inner ear have not been elucidated. For this purpose, we developed a three-dimensional (3D) finite element model based on Fick's second law of diffusion to quantitatively predict the diffusion profile of BDNF using a microfluidic neural culture system (XONA, Xona Microfluidics, LLC, Research Triangle Park, NC, USA). The reaction kinetics of BDNF in the PODS crystals and its subsequent degradation were assessed; concentration-time data were analyzed using a MATLAB curve-fitting algorithm to obtain constants for the mathematical model. Using COMSOL Multiphysics Software (COMSOL, Burlington, MA), a time-dependent study was run with varying initial parameters to simulate the diffusion kinetics of BDNF in the PODS crystals through the 3D surface of a XONA microfluidic chip. Three-dimensional diffusion was modeled graphically prior to experimentation to optimize initial BDNF-PODS concentrations. The mathematical model predicted that 20,000 BDNF-PODS crystals would provide a BDNF concentration sufficient for sustained neurite growth in hPSC-derived otic neuronal progenitors over a 7-day period. Following the culture period, immunocytochemistry was performed to assess neurite growth and otic neuronal differentiation. Preliminary results indicated that neurite extension was oriented toward the BDNF-PODS crystals and that neurite length was proportional to the number of BDNF-PODS crystals. The results from the empirical experiments can be compared with the parameters and coefficients of the mathematical model for further insights and improved iterations.

Keywords: Inner ear regeneration, Diffusion kinetics, Microfluidics

Poster: 828

HUMAN CEREBRAL ORGANOIDS OFFER MULTIMODAL SCREENING OPTIONS FOR THERAPEUTICS TARGETING INFECTIOUS PRION DISEASES

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Prion diseases are infectious, fatal and currently incurable neurodegenerative conditions. Many prospective treatments have proven promising in mouse models of disease but have failed to translate into effective treatments when trialed in humans. Our understanding of why these therapies have failed has been restricted by the lack of a human model in which to test them. Recently we developed a human cerebral organoid model of prion disease. To determine if this model could be used to evaluate the efficacy of putative therapeutics, we used pentosan polysulfate (PPS), a known anti-prion compound, to assess the reduction of prion infection within the organoids. PPS has been used to treat patients with prion disease, but its usefulness is restricted by its inability to cross the blood brain barrier, instead requiring an intraventricular pump. Cerebral organoids in culture do not suffer the limitation of the blood brain barrier so PPS could be used as a model compound. Two modes of treatment were assessed; a treatment intended to mimic prophylaxis, as might be required upon accidental exposure to infection, and a treatment designed to represent therapeutic use that would be required post-symptom onset in a presenting patient. Experimental readouts of prion infection included detection of seeding activity (demonstrating the capacity of prions to propagate), accumulation of protease-resistant mis-folded prion protein and deposition of prion protein within the organoid core. As compared with sham treated controls, both treatment paradigms showed that PPS could reduce the experimental measurements of disease with no toxicity to the tissue. We conclude that cerebral organoids have the capacity to identify putative therapeutic compounds that can reduce prions in human brain tissue, and can be used for screening differing treatment paradigms that reflect how a target therapeutic might be used in the clinic.

Funding Source: This research was supported by the Intramural Research Program of the NIH (NIAID) as well as by a CJD Foundation grant awarded to Natalia C. Ferreira.

Keywords: Prion, Neurodegeneration, Human cerebral organoid

Poster: 829

AFFINITY-CONTROLLED DELIVERY OF THERMO-STABILIZED CHONDROITINASE ABC USING CROSS-LINKED METHYLCELLULOSE HYDROGELS AS A THERAPEUTIC STRATEGY FOR STROKE

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Stroke is one of the leading causes of mortality and long-term disability worldwide, with no current treatment to regenerate lost brain tissue. Cell transplantations were proposed as a treatment option, demonstrating improved functional behavior in pre-clinical studies, yet cell survival and integration remain poor. One crucial obstacle is the loss of tissue plasticity, restricted by the formation of a proteoglycan-rich glial scar. Degradation of the scar was therefore suggested as a therapeutic strategy to promote local plasticity and axonal regrowth. Bacterial 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, limits its therapeutic potential. Moreover, prolonged delivery is needed to obtain significant improvements after a stroke. We previously reported an affinity-controlled delivery platform for ChABC, in which the enzyme is expressed as a fusion protein with a Src homology-3 (SH3) domain and encapsulated in an injectable cross-linked methylcellulose hydrogel modified with SH3 binding peptides. To address the intrinsic instability of ChABC, we designed a thermo-stable, 37 mutation, ChABC (ChABC-37-SH3) using computational remodeling and then tested it experimentally. The ChABC-37-SH3 demonstrated a 6.5 times longer half-life than the native enzyme, with a higher melting temperature, and increased activity for its substrate. We are now, for the first time, evaluating a minimally invasive, sustained release strategy of

this thermo-stabilized enzyme in vivo, using an endothelin-1-induced stroke injury model in rats. The in vivo efficacy of this strategy will be described in terms of tissue benefit, including mechanism of action and degradation of the glial scar.

Funding Source: We are grateful to NSERC and CIHR from CHRP for funding this research.

Keywords: Stroke, Glial scar, Chondroitinase ABC

Poster: 830

HUMAN INHIBITORY NEURON CELL THERAPY FOR CHRONIC FOCAL EPILEPSY

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Mesial temporal lobe epilepsy (MTLE) is the most common type of focal epilepsy. In MTLE, seizures typically originate from a sclerotic hippocampus. Approximately one-third of patients do not adequately respond to anti-seizure drugs and have few effective therapeutic options. Surgery to remove or ablate the affected temporal lobe is one such option, but is not indicated or effective for all individuals and can have adverse effects. Here, we report the development of a cell therapy alternative to resection/ablation surgery for drug-resistant MTLE. The cell therapy candidate comprises cryopreserved post-mitotic inhibitory interneurons derived from a clinical-grade human embryonic stem cell line using a reproducible, cGMP-compatible manufacturing process. Following cell thaw and intra-hippocampal delivery in a chronic mouse model of pharmacoresistant MTLE, seven independently produced lots of human interneurons resulted in consistent and stable suppression of electrographic seizures, with 58% of animals becoming seizure-free and 70% of animals with >75% seizure reduction versus vehicle-treated control animals. The grafted human interneurons locally dispersed, matured into cortical/hippocampal-type interneurons, and persisted in the damaged mouse hippocampus throughout the 9-month study. Characteristic hallmarks of MTLE, such as hippocampal granule cell dispersion and degeneration, were remarkably reduced after administration of the human interneurons. Suppression of both seizure activity and neuropathology was dose-dependent and suggested a broad therapeutic dosing range. No ectopic tissue or teratoma formation was detected. Furthermore, behavioral abnormalities were not observed at any dose on a battery of tests of general well-being, locomotion, and anxiety. Thus, these findings support human interneuron cell therapy to restore local inhibitory tone for the potential treatment of drug-resistant focal epilepsies.

Funding Source: This research was made possible in part by a grant from the California Institute for Regenerative Medicine (Grant Number TRAN1-11611).

Keywords: Epilepsy, Cell therapy neuron transplant, MGE GABA interneuron

Poster: 831

SARS-COV-2 INFECTION & TREATMENT OF CEREBRAL ORGANOIDS DIFFERS FROM THAT OF LUNG ORGANOIDS

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COVID-19 is a disease known to infect cells of the proximal & distal tracheobronchial tree, canonically entering the cell via adherence of its spike protein to the ACE2 receptor, abetted by the protease, TMPRSS2. There is growing evidence that COVID-19 has an effect on the CNS, though there is ongoing debates as to which cell types in the CNS are directly infected, if at all. Clearly, discrepant findings from different groups need to be reconciled additional research on which & how CNS cells are impacted by SARS-CoV-2 which will help inform how such infection may be averted and/or treated. We have been utilizing cerebral organoids derived from hiPSCs as well as primary human fetal brain which are then infected with various strains of live, replication competent SARS-CoV-2 at varying MOIs and time points to analyze the target(s) of infection using scRNAseq and proteomic analysis. Complete lung organoids were infected & treated in parallel as positive controls. To analyze treatment options, we have been systematically testing drugs that block the iterative stages of cell entry & viral life cycle, including inhibitors of protease-mediated viral entry, inhibitors of non-protease-mediated late endocytosis & post-entry events, inhibitors of viral replication, as well as blocking multiple viral entry routes & life cycle events simultaneously (a potential basis for synergistic drug combinations). Data to date has shown some reduction by these drugs in infection (as assayed by the presence of nucleoprotein), but much less than in the lung organoids, suggesting that SARS-CoV-2 may be impacting the CNS by non-canonical routes, different from those it employs in the lung.

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Keywords: COVID-19, Cerebral Organoid, SARS-CoV-2

Poster: 832

FUNCTIONAL ENDPOINTS FROM HUMAN IPSC-DERIVED SENSORY NEURONS FOR PAIN DRUG DISCOVERY

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Drug discovery workflows for neurological indications have traditionally relied on neuronal preparations from rodent tissues. However, the ability to test activity of compounds on human neurons is desirable, since rodent pharmacology

data do not always predict effects in humans. Human iPSC-derived neurons are increasingly used to provide human in vitro pharmacology data, with the promise of improving decision-making for neuroscience drug discovery. In pain research, human iPSC derived sensory neurons have been used by recent studies to model primary sensory neurons of dorsal root ganglia. Although different types of human iPSC-derived neurons have been extensively characterized by gene expression, the most salient phenotype of neurons is electrical excitability, as neurons integrate synaptic inputs and respond by firing action potentials. Patch-clamp electrophysiology assays are routinely used to characterize neuronal excitability and provide functional endpoints to assess effects of drug compounds. However, the basic electrophysiological properties of human iPSC-derived sensory neurons have not been well studied. How well do iPSC-derived sensory neurons reflect the physiological state of native primary sensory neurons? How consistent is the phenotype? How reproducible are the data? To address these questions, we focused on human sensory neurons differentiated from iPSC-derived neural progenitors (Axol Bioscience, ax0055). We conducted a thorough patch-clamp analysis, including: 1) current-clamp recordings to measure spontaneous and evoked action potential firing and 2) voltage-clamp recording to characterize tetrodotoxin-sensitive and tetrodotoxin-resistant Na currents (found in native neurons). We also determined the concentration-response of tetracaine, a voltage- and frequency-dependent blocker of voltage gated Na currents. We recorded >500 neurons from twelve independent neural precursor differentiation experiments. We conclude that human iPSC-derived sensory neurons reproduce several key electrophysiological properties expected from bona fide dorsal root ganglia sensory neurons and are useful for supporting drug discovery programs for pain.

Keywords: PAIN, SENSORY NEURONS, DRUG DISCOVERY

Poster: 833

ELECTROPHYSIOLOGICAL EVIDENCE OF FUNCTIONAL INTEGRATION OF HUMAN BRAIN ORGANOIDS WITH THE RODENT VISUAL NETWORK

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Due to limitations in the brain's inherent repair mechanisms, brain injury often results in lifelong neurological deficits. One approach to augmenting the brain's neuroregenerative capacity is through the transplantation of brain organoids derived from human induced pluripotent stem cells (iPSC). Recent studies have demonstrated robust survival of organoid grafts and evidence of graft connectivity with local host neurons. However, studies showing the potential of brain organoids to integrate with systems-level circuitry of the host brain are lacking. To study this, forebrain organoids expressing green fluorescent protein were generated from a human iPSC line. D80 organoids were transplanted into adult male Long Evans rats after an aspiration lesion was created in visual cortex (N=14). 1 or 2 months after transplantation, animals were re-anesthetized, and the organoid graft was surgically exposed. Multi-electrode laminar probes were inserted into the transplanted organoid using the graft's fluorescent signal for guidance, and in vivo electrophysiologic recordings from organoid grafts and naïve visual cortex were compared. Spontaneous activity recordings were followed by 2 stimulation paradigms: a flashing screen and drifting gratings. 95 single units were identified in organoid grafts in 5 rats. There were no significant differences in firing rate or spike amplitude between organoid units and naïve brain units, but naïve brain units had significantly greater spike widths. Two of the five organoids showed units that were responsive to the flashing screen. Latency to peak firing rate was not significantly different between evoked units in organoid grafts and naïve visual cortex. Event-related potentials (ERPs) in the organoid tissue had significantly smaller amplitudes than ERPs in the native brain. The presence of stimulus-responsive units and evoked fields in transplanted organoids provides the first evidence that structured neural tissue transplants can functionally integrate with native brain circuity. These findings support the possibility that human pluripotent stem cell-derived organoids can be an effective intervention to reverse the deficits associated with brain injury.

Funding Source: IK2RX002013 from VA RR&D

Keywords: cortical organoid transplantation, in vivo electrophysiology, visual processing

Poster: 834

MESENCHYMAL STEM CELL-NEURAL PROGENITORS ARE ENRICHED FOR CELL SIGNALING MOLECULES AND MEDIATORS OF CELL MIGRATION IMPLICATED IN THEIR THERAPEUTIC EFFECT IN MULTIPLE SCLEROSIS

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Mesenchymal stem cell-derived neural progenitors (MSC-NP) are a subpopulation bone marrow MSCs with neuroectodermal characteristics and reduced mesodermal differentiation potential. Previous studies have shown that MSC-NPs secrete immunomodulatory and trophic factors that act in a paracrine manner to promote neural repair. Phase II clinical investigation of intrathecal injection of autologous MSC-NPs is currently underway as a regenerative cell therapy in patients with secondary progressive (SP) and primary progressive (PP) multiple sclerosis (MS). As this cell therapy moves into clinical use, there is a need to better define and characterize MSC-NPs in order to better understand their therapeutic mechanisms. The objective of this study was to define the transcriptional profile of MSC-NPs from MS and non-MS donors in order to identify their functional characteristics and therapeutic potential. MSCs were derived from 8 donors (SPMS n=3; PPMS n=3; non-MS n=2) and cultured under standard conditions in media containing human platelet lysate. MSC-NPs were generated from MSCs by culturing in serum-free media containing EGF and bFGF. RNA from each population of MSCs and MSC-NPs was sequenced and differentially expressed genes were determined. Gene ontology analysis was performed using GORilla. MSCs derived from SPMS, PPMS, or controls demonstrated minimal differential gene expression despite differences in disease type, duration, and donor age. Compared to all MSCs, MSC-NPs exhibited significant differential gene expression with 2,156 and 1,467 genes upregulated and downregulated, respectively. Gene ontology analysis demonstrated pronounced downregulation

in cell cycle genes in MSC-NPs compared to MSCs, which was confirmed by lack of BrdU incorporation in MSC-NPs. In addition, MSC-NPs demonstrated significant enrichment of genes involved in the regulation of cell-to-cell communication, humoral immune response, cell migration, and neuronal differentiation. Increased release of secreted molecules including HGF, TIMP1, TGFß, and complement C3 were validated independently and their relationship to therapeutic potency of MSC-NPs was investigated in vitro. These findings suggest that increased cell signaling and chemotactic capability of MSC-NPs support their therapeutic efficacy in MS.

Keywords: mesenchymal stem cells, multiple sclerosis, RNA sequencing

Poster: 835

UMBILICAL CORD MESENCHYMAL STEM CELLS TREATMENT IS ASSOCIATED WITH IMPROVED OUTCOMES IN PATIENTS WITH COVID-19 ARDS IN A DOUBLE-BLIND, PHASE 1/2A, RANDOMIZED CONTROLLED TRIAL.

Stone, Logan D.[1], Poggioli, Raffaella[1], Alvarez Gil, Ana[1], Baidal, David[1], Metalonis, Sarah[2], Alvarez, Roger Argelio[3], Marttos, Antonio[3], Pastewski, Andrew[4], Bell, Crystal Ann[5], Kusack, Halina[5], Kouroupis, Dimitrios[1], Ruiz, Phillip[6], Hirani, Khemraj[1], Rafkin, Lisa Emily[1], Gawri, Kunal[3], Lenero, Clarissa[1], Mantero, Alejandro Max Antonio[2], Wang, Xiaojing[1], Xu, Xiumin[1], Cayetano, Shari Messinger[2], Linetsky, Elina[1], Alejandro, Rodolfo[1], Ricordi, Camillo[1], Lanzoni, Giacomo[1]

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COVID-19 is a pneumonia-like disease caused by infection from the SARS-CoV-2 coronavirus. In a subset of patients, COVID-19 becomes severe: an excessive pro-inflammatory response can cause cytokine storm and Acute Respiratory Distress Syndrome (ARDS). COVID-19 ARDS is associated with high morbidity and mortality. Mesenchymal Stem Cells exert robust immunomodulatory and anti-inflammatory effects, and could thus be beneficial in COVID-19 ARDS. The objective of this study was to investigate safety and explore efficacy of Umbilical Cord-derived Mesenchymal Stem Cells (UC-MSC) infusions in subjects with COVID-19 ARDS. A double-blind, phase 1/2a, randomized controlled trial was performed. Randomization and stratification were based on ARDS severity. Twenty-four subjects were randomized to either UC-MSC treatment (n=12) or control (n=12) group. Subjects in the UC-MSC group received two intravenous infusions at Day 0 and Day 3 (100 x 10e6 cells). Controls received two intravenous infusions of vehicle solution at the same timepoints. Both groups also received standard of care. Disease severity was analyzed using the W.H.O. COVID-19 ordinal scale at all study visits up until 90 days. One subject was censored on Day 4 due to failed intubation, one subject was censored after Day 6 due to loss to follow-up, and one subject missed the day 14 follow up but was found fully recovered on day 31. Ordinal scale scores were stratified as follows: no oxygen therapy, non-invasive oxygen therapy, mechanical ventilation, and death. On Day 31, there was a significant difference between the UC-MSC treatment and control group based on ordinal scale stratification (P=0.047, Wilcoxon rank sum test): there were 8 vs 5 patients with no oxygen therapy, 1 vs 0 on mechanical ventilation, and 1 vs 7 deaths in UC-MSC vs control group, respectively. UC-MSC treatment was associated with significant clinical improvement. Treatment was associated with significantly improved patient survival (91% vs 42%, P =.015), Serious Adverse Event-free survival (P =.008), and time to recovery (P =.03) (Kaplan-Meier estimates). UC-MSC infusions are safe and could be beneficial in treatment of subjects with COVID-19 ARDS. A larger trial has been designed to further assess the beneficial role of UC-MSC in improving severe COVID-19 outcomes.

Funding Source: The Cure Alliance; North America's Building Trades Unions; National Center for Advancing Translational Sciences; Ugo Colombo; Simkins Family Foundation; Fondazione Silvio Tronchetti Provera; Barilla Group and Family

Keywords: COVID-19, Acute Respiratory Distress Syndrome (ARDS), Umbilical Cord Mesenchymal Stem Cells, UC-MSC

Poster: 1018

REPROGRAMMING ENRICHES FOR SOMATIC CELL CLONES WITH SMALL SCALE MUTATIONS IN CANCER-ASSOCIATED GENES

Kosanke, Maike[1], Osetek, Katarzyna[1], Haase, Alexandra[1], Wiehlmann, Lutz2, Davenport, Colin[2], Schwarzer, Adrian[3], Adams, Felix[3], Kleppa, Marc-Jens[3], Schambach, Axel[3], Merkert, Sylvia[1], Wunderlich, Stephanie[1], Menke, Sandra[1], Dorda, Marie[2], Martin, Ulrich[1]

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Cellular therapies based on induced pluripotent stem cells (iPSCs) come out of age and an increasing number of clinical trials applying iPSC-based transplants are ongoing or in preparation. Recent studies, however, demonstrated a high number of small scale mutations in iPSCs. Although, the mutational load in iPSCs seems to be largely derived from their parental cells, it is still unknown whether reprogramming may enrich for individual mutations that could lead to loss of functionality and tumor formation from iPSC derivatives. 30 hiPSC lines were analyzed by whole exome sequencing. High accuracy amplicon sequencing showed that all analyzed small scale variants pre-existed in their parental cells and that individual mutations present in small subpopulations of parental cells become enriched among hiPSC clones during reprogramming. Among those, putatively actionable driver mutations affect genes related to cell cycle control, cell death and pluripotency, and may confer a selective advantage during reprogramming. Finally, a shRNA-based experimental approach was applied to provide additional evidence for the individual impact of such genes on the reprogramming efficiency. In conclusion, we show that enriched mutations in curated onco- and tumor suppressor genes may account for an increased tumor risk and impact the clinical value of patient-derived hiPSCs.

Keywords: Induced pluripotent stem cells, Genomic integrity, Small scale mutations

SATURDAY, JUNE 26

00:00 - 1:00 EDT

POSTER SESSION 9

CELLULAR IDENTITY

Poster: 901

GAP JUNCTION WITH MLO-A5 OSTEOBLAST-LIKE CELLS INDUCE ALP AND BSP TRANSCRIPTION OF 3T3-L1 PRE-ADIPOCYTE-LIKE CELLS VIA HSPB1 WHILE RETAINING ADIPOGENIC DIFFERENTIATION ABILITY

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Previously, it had been demonstrated that transcriptions of alkaline phosphatase and bone sialoprotein), which are osteoblast markers, were induced in C3H10T1/2 murine mesenchymal stem cell line, when they were co-cultured with MLO-A5 murine osteoblast-like cell line. However, the molecular mechanisms underlying this process has not been fully understood. Moreover, few advances have been made toward understanding the communication networks that link the status of committed cells such as (pre-) adipocytes that differentiated from mesenchymal stem cells as well as osteoblasts. Therefore, we attempted the present study to elucidate the mechanism of the communication network between pre-adipocytes and osteoblasts. We evaluated the effect of co-culture with osteoblast on the cell status of pre-adipocytes using murine osteoblast-like cell line, MLO-A5, and pre-adipocyte-like cell line, 3T3-L1, respectively. Our results presented here indicated that osteoblasts and pre-adipocytes communicate via gap junctions, and the ensuing drastic increase in alkaline phosphatase and bone sialoprotein transcription in co-cultured pre-adipocytes was induced, at least partly, via heat shock protein family B member 1 (Hspb1). Furthermore, terminal differentiation into adipocytes was inhibited in pre-adipocytes during co-culture with osteoblast without loss of adipogenic differentiation ability. Interestingly, after co-culture with osteoblasts, isolated co-cultured pre-adipocytes were able to differentiate to adipocytes as well as original pre-adipocytes. These results suggest that gap junctional communication with osteoblasts suppressed adipogenic differentiation of pre-adipocytes without loss of adipogenic differentiation ability.

Keywords: preadipocyte, osteoblast, gap junction

Poster: 902

CYTOCHALASIN B MODULATES NANOMECHANICAL PATTERNING AND FATE IN HUMAN ADIPOSE-DERIVED STEM CELLS

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Cytoskeletal proteins provide architectural and signaling cues within the cells. They are able to reorganize themselves in response to mechanical forces, converting the stimulus received into specific cellular responses. Thus, the cytoskeleton influences the cell shape, its proliferation and even differentiation. In particular, the cytoskeleton affects the fate of mesenchymal stem cells (MSCs), highly attractive candidates for cell therapy approaches due to their capacity for self-renewal and multi-lineage differentiation. Cytochalasin B (CB), a cyto-permeable mycotoxin isolated from an ascomycete fungus, is able to inhibit the formation of actin microfilaments with direct effects on cell properties. Here we investigate for the first time the role of CB on human adipose-derived stem cells (hADSCs) both after toxin treatment at different doses and after a recovery time post CB administration. CB showed the ability to influence cell viability, morphology, number, metabolism and adipogenic and osteogenic differentiation, in a dose-dependent manner, in association with a progressive disorganization of actin. Finally, atomic force microscopy (AFM) revealed that CB remarkably modulated the viscoelastic properties of hADSCs, by reducing their stiffness, while increasing their viscosity.

Funding Source: This research was funded by Di Bella Foundation, Via Guglielmo Marconi 51, 40122 Bologna, Italy and the Eldor Lab, via Vittor Pisani 16, 20124 Milan, Italy.

Keywords: Cytoskeleton, Human adipose-derived stem cells, Atomic Force Microscopy

Poster: 903

ROLE OF HIPPO PATHWAY IN REGULATING PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS ON SOFT SUBSTRATE

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Growth factors and chemical cues are well-known regulators of stem cells, but recent studies demonstrated the role of biophysical signals in regulating stem cell proliferation and differentiation through mechanotransduction pathways. Hippo pathway member -transcriptional coactivator Yes-associated protein (YAP) conveys the mechanical signals to the nucleus. Cytoskeletal tension and reorganization of F-actin bundles lead to post-translational modifications of YAP by upstream Hippo pathway components, this leads to substrate-dependent changes; however, how YAP regulates lineage specification in human pluripotent stem cell differentiation has not been demonstrated. Most of the published literature has shown that mouse ESCs and human mesenchymal stem cells differentiate in response to changes in substrate stiffness alone. We cultured hESCs on the substrate of varying stiffness firstly in media that supports pluripotency and later in media that encourages differentiation; followed by characterization of markers that define pluripotency and lineage specification. Importantly, we investigated how changing substrate stiffness affected YAP, and how this correlated with pluripotency or differentiation. Contrary to findings reported with human mesenchymal stem cells, our findings showed, in pluripotency supporting media, varying substrate stiffness did not induce differentiation in hESCs; thus, implying some fundamental difference between how these stem cells respond to changes in their environment. It has been previously reported that a decrease in YAP expression causes loss of pluripotency in mouse ESCs, however, our findings revealed that hESCs maintain a minimum basal level of YAP expression for cell survival and proliferation, but YAP might not correlate directly with pluripotency. We further demonstrate the effect of YAP inhibition by a pharmacological inhibitor on hESCs proliferation and differentiation when cultured on the substrate with varying stiffness. Our novel findings clearly show that biochemical cues and substrate stiffness are interdependent and play an important role in cell differentiation.

Funding Source: This work was funded by the Department of Biotechnology (BT/PR28474/MED/31/393/2018), Govt. of India.

Keywords: Human Embryonic Stem Cell Pluripotency, Mechanobiology, HIPPO pathway

Poster: 904

POLYCOMB REPRESSIVE COMPLEX 2 COORDINATES TOTAL GLYCOME DYNAMICS DURING THE MOUSE NAÏVE-TO-PRIMED PLURIPOTENCY STATE TRANSITION

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Embryonic stem cells (ESCs) are established from the inner cell mass in pre-implantation embryos at E3.5-E4.52. Epiblast-like cells (EpiLCs), which differentiate from ESCs in culture, resemble the post-implantation stage at E5.5-E6.53. ESCs and EpiLCs reflect two distinct pluripotent states known as the naive state and the primed state, respectively. To date, a large number of studies have investigated the changes underlying the transition from the naïve state to the primed state of pluripotency using mouse EpiLCs and mouse ESCs. Nevertheless, a significant amount of attributes still remain unexplored. Glycosylation is a multi-step post-translational modification and is involved cellular processes, such as adhesion, signaling regulation, endocytosis, protein folding and protein stability. It has been reported that glycosylation plays a pivotal role in development and stem cell pluripotency regulation. However, a comparative analysis of glycomes during the naïve-to-primed transition is currently missing. In this study, we performed a comparative and comprehensive analysis of glycomes of mouse ESCs and EpiLCs, demonstrating that glycosylation undergoes dramatic alterations from early stages of development. Together with the identification of novel specific structures across all glycosylation classes, hence providing additional markers to distinguish between the naïve and primed pluripotent states, we showed for the first time the presence of a developmentally regulated network orchestrating glycosylation changes and identified polycomb repressive complex 2 (PRC2) as a key component involved in this process. Indeed, inhibition of PRC2 in mouse ESCs resembled, both structurally and transcriptionally (43% of total glycosylation related genes), the glycosylation state observed in mouse EpiLCs. Our findings provide the first line of evidence supporting the presence of a regulatory network orchestrating glycosylation dynamics during developmental transitions.

Funding Source: This work was supported by JSPS KAKENHI Grant Number JP18K06139 (to S.N.) and JST-Mirai Program Grant Number JPMJMI18GB (to S.N.), Japan, research funds from SEI (to J.F.), and the NBDC of the JST (to S.O.).

Keywords: pluripotency, PRC2, glycosylation

Poster: 905

NUCLEAR M6A READER YTHDC1 REGULATES THE SCAFFOLD FUNCTION OF LINE1 IN MOUSE EMBRYONIC STEM CELLS

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N6-methyladenosine (m6A) on chromosome-associated regulatory RNAs (carRNAs), including repeat RNAs, play important roles in tuning the chromatin state and transcription. Among diverse RNA-chromatin interacting modes, the nuclear RNA scaffold is considered important for trans-interactions but has not yet been connected with m6A yet. Here, we found that YTHDC1 played indispensable roles in the embryonic stem cell (ESC) self-renewal and differentiation potency, and these roles highly depended on its m6A-binding ability. Ythdc1 deficiency in ESCs resulted in decreased rRNA synthesis and the activation of 2-cell (2C) embryo-specific transcriptional program, and these observations recapitulated the transcriptome defects induced by dysfunction of the long interspersed nuclear element-1 (LINE1)-scaffold, which were unrelated to the direct targeting of YTHDC1. A detailed analysis revealed that YTHDC1 recognized

m6A on LINE1 and was physically involved in the formation of the LINE1-NCL-KAP1 partnership. In summary, our study reveals a new link between m6A and the RNA scaffold and thus provides a new regulatory model for the crosstalk between RNA and the chromatin epigenome.

Keywords: YTHDC1, LINE1-scaffold complex, 2C state

Poster: 906

NUCLEOSOME RECONFIGURATION IN MOUSE SOMATIC CELL NUCLEAR TRANSFER EMBRYOS

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Somatic cell nuclear transfer (SCNT) can reprogram terminally differentiated somatic cells into totipotent embryos. However, the birth rate of SCNT embryos is extremely low compared with that of fertilized embryos. But the underlying molecular mechanisms remain unclear. The nucleosome, which is the fundamental structural unit of chromatin in eukaryotes, is closely related to gene expression. How nucleosome position change after the donor cell nuclear transferred into enucleated oocytes is largely unexplored. Here, we mapped genome-wide profiling of nucleosome occupancy and positioning in early mouse SCNT embryos using Low-input MNase-seq. We observed the nucleosome depletion regions (NDRs) around TSS regions disappear as early as 1 hour after the nuclear injection and re-established until 6 hours after activation of the SCNT embryos, which indicated nucleosome position undergo dramatic changes in early SCNT embryos. Dynamic of nucleosome position occurred in SCNT embryos were different from those in fertilized embryos. Subsequently, we defined differentially expressed genes between donor cells and 2-cell embryos or ICM derived from blastocyst and found the gene expression level of 2-cell or ICM is positively correlated with the NDR score around TSS regions in donor cells. These results indicated that aberrant nucleosome occupancy at promoters and enhancers in SCNT embryos is a potential cause of its developmental defects. Our study provide insight into nucleosome reconfiguration during early SCNT embryo development.

Keywords: SCNT, nucleosome, reprograming

Poster: 907

EFFECTS OF NEUROTROPHIN-4 ON IN VITRO MATURATION OF PORCINE OOCYTES

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Neurotrophin-4 (NT-4) is a member of neurotrophin family and is a growth factor that play crucial roles in oocyte maturation and follicular development. Here, we investigated the effect of NT-4 treatment during porcine oocytes in vitro maturation (IVM) and subsequent embryonic development after parthenogenetic activation (PA). During IVM, the defined maturation medium was supplemented with various concentrations of NT-4 (0, 1, 10, and 100 ng/mL). After 44 h of IVM, the nuclear maturation rate significantly increased in the 10- and 100 ng/mL NT-4 treated groups (89.0 ± 0.5 and 89.6 ± 0.7%, respectively) than control (0 ng/mL of NT-4 group; 85.3 ± 1.8%). We also measured the intracellular glutathione (GSH) levels and reactive oxygen species (ROS) levels after 44 h of IVM. There was no significant difference in the intracellular ROS levels between control and all NT-4 treatment groups, but the 1- and 10 ng/mL NT-4 treated groups (1.040 ± 0.01 pixels/oocytes and 1.036 ± 0.01 pixels/oocytes, respectively) showed a significant increase in the intracellular GSH levels compared with control (1.000 ± 0.01 pixels/oocytes). In cumulus cells, the 10 ng/mL treatment group showed significantly increased cumulus expansion-related genes (HAS2 and TNFAIP6). In matured oocytes, the 10 ng/mL treatment group significantly increased the cell proliferation-related genes (PCNA and EGFR), and the antioxidant-related gene (NRF2). In addition, we examined the developmental competence of NT-4 treated oocytes during IVM after parthenogenetic activation (PA). There was no significant difference of cleavage rates, but only the 10 ng/ml NT-4 treated group showed a significantly higher blastocyst formation rate and total cell numbers than control (59.9 ± 3.1 % and 71.4 ± 2.5 vs 41.5 ± 3.0 % and 67.4 ± 4.9, respectively). Taken together, these results showed that 10 ng/mL NT-4 supplementation during IVM could improve nuclear and cytoplasmic maturation of porcine oocytes and promote subsequent developmental potential of PA 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, 819029-2)", Republic of Korea.

Keywords: Neurotrophin-4, In vitro maturation, Pig

Poster: 908

TREHALOSE IMPROVED THE DEVELOPMENT POTENTIAL OF PORCINE PARTHENOTES VIA DECREASE EMBRYONIC FRAGMENTATION AND APOPTOSIS

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Autophagy is a crucial process for maternal to zygotic transition in numerous species embryonic development. Additionally, the previous study mentioned that osmolality also influences the viability of embryonic development. Trehalose, a natural disaccharide, is known to stimulate autophagy via an mTOR-independent manner and also to be able to mediate the osmotic pressure. So far, the role of trehalose on porcine embryonic development potential still is an open question. To address this, the embryonic development competence, total cell number, and apoptotic index of blastocysts were evaluated after performed parthenogenetic activation. We designed experimental groups by various concentrations (0, 25, 50, 100, and 200 mM) and durations (0, 12, 24, 48, and 72 hours), respectively, to determine the optimal condition of trehalose supplementation. Results in the present study indicated that 100 mM trehalose treated during 12-48h show the best effects as the rates of cleaved embryos (77.08% ± 4.04, 87.37% ± 4.27, and 90.53% ± 1.38 vs. 57.29% ± 3.02) and formatted blastocysts (55.79% ± 10.73, 58.95% ± 3.49, and 67.37% ± 2.11 vs. 33.68% ± 3.02) were significantly ($P < 0.05$) increased compare to the control. Furthermore, the number (2.85 ± 0.31, 1.98 ± 0.19, and 2.65 ± 0.24 vs. 3.91 ± 0.76) and index (3.89% ± 0.44, 2.83% ±

0.33, and 3.56% ± 0.47 vs. 5.42% ± 0.91) of apoptotic nuclei were significantly ($P < 0.05$) decreased versus the control. In addition, the results showed that trehalose supplementation more than 48h delayed mitotic division as the rate of 4-5 cell stage was dramatically ($P < 0.05$) higher in 72h treated group than control in day 6, and the rate of 9 cell-morula stages of embryos in 48h and 72h treated groups were statistically ($P < 0.05$) higher than control in day 5 and 6. In summary, we demonstrated that trehalose treatment during in vitro culture increased the viability of porcine embryonic development via decreasing embryonic fragmentation and protecting embryos from apoptosis.

Keywords: Trehalose, embryo, apoptosis

Poster: 909

H3K9ME3 AT NANOG IS DETERMINANT FOR EFFICIENT COMMITMENT TOWARDS PRIMITIVE ENDODERM FATE IN MOUSE

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Nanog is one of the master transcription factors supporting pluripotency in Embryonic Stem (ES) cells and to differentiate, ES cells need to silence Nanog expression. Extrinsic signaling cues, such as FGF/ERK and GSK3b activity, have been shown to downregulate Nanog, both in undifferentiated cells – leading to wide expression fluctuations of Nanog –, and during differentiation when Nanog is irreversibly silenced. Here, we show that ERK activity leads to histone H3 lysine 9 trimethylation (H3K9me3) at the Nanog locus, between its promoter and its 5kb-upstream enhancer. Using a range of molecular and cellular assays upon the deletion of the region, our results suggest that ERK-dependent H3K9me3 at Nanog stabilizes the Nanog negative state in undifferentiated cells and locks down Nanog expression upon exit of pluripotency, thereby modulating the timing of commitment into differentiation and, more strikingly, enabling effective Primitive Endoderm specification.

Funding Source: Institut Pasteur & LabEx Revive

Keywords: Nanog, H3K9me3, PrE

Poster: 910

MIXL1 ACTIVATION REMODELS CHROMATIN AT LINEAGE SPECIFIC ENHANCERS TO POISE EPIBLAST STEM CELLS TOWARDS ENDODERM SPECIFICATION

Salehin, Nazmus, Santucci, Nicole, Knowles, Hilary, Li, Bella, Osteil, Pierre, Tam, Patrick PL

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During mouse gastrulation, the transcription factor MIXL1 is vital for mesoderm and definitive endoderm germ layer specification. Mouse Mixl1 knock-out models fail to progress past gastrulation while cells without functional MIXL1 do not contribute to definitive endoderm lineages in mouse chimera studies. In vitro studies using mouse embryonic stem cells showed activation of Mixl1 resulted in an increased efficacy of mesoderm and endoderm formation. More recently, within mouse epiblast stem cells (EpiSC) subjected to undirected differentiation, endodermal specification appeared to be a correlated to the timing and robustness of Mixl1 expression. to identify endoderm specification In a novel EpiSC model of Mixl1-timed activation, we show that modulation of the timing of Mixl1 influences the proportion of CXCR4-positive definitive endoderm cells at the end of undirected differentiation – early induction of Mixl1 during undirected differentiation results in the greatest proportion of definitive endoderm. To explore the functional role of Mixl1 during this narrow window, we performed bulk ChIP-seq, RNA-seq and ATAC-seq studies of differentiating cells immediately after early Mixl1 activation. MIXL1 was found to act, at an evolutionarily conserved double ATTA motif, as a transcriptional activator of genes implicated in mesendoderm formation and repressor of ectoderm lineage genes. Furthermore, the induction of Mixl1 results in an increase in the accessibility of mesendoderm specific enhancers and regions harbouring double ATTA motifs, and a decrease in that of ectoderm specific enhancers and regions harbouring motifs for POU and SOX transcription factors. Taken together, these results suggest that the timing of Mixl1 expression influences germ layer specification during gastrulation by direct transcriptional activation and downstream chromatin remodelling of lineage specific enhancers. Our efforts now turn to perturbation screening of direct downstream targets of MIXL1 to determine the regulome of MIXL1 and the genetic cascade critical for MIXL1 action during endoderm specification.

Funding Source: This research was supported (partially or fully) by the Australian Government through the Australian Research Council's Discovery Projects funding scheme (project DPDP160103651).

Keywords: gastrulation, epiblast stem cell, endoderm

Poster: 911

REPROGRAMMING OF H3K9ME3-MEDIATED HETEROCHROMATIN DURING THE PRE-IMPLANTATION DEVELOPMENT OF MOUSE SCNT EMBRYOS

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Somatic cells can be reprogrammed into a pluripotent state through somatic cell nuclear transfer (SCNT), yet exhibiting extremely low efficiency. Donor-deposited H3K9me3 is deemed to be an epigenetic barrier impeding normal genomic activation in 2-cell cloned embryos. However, the dynamic organization of H3K9me3-mediated heterochromatin during early SCNT embryogenesis remains elusive. Here, we generated genome-wide reprogramming profiling of H3K9me3 during the pre-implantation development of mouse cloned embryos. Although the holistic loss of donor-derived H3K9me3 was found shortly after activation, persistently redundant H3K9me3 deposition was observed throughout early cloned embryo development in comparison to fertilized embryos. Interestingly, CGI-biased de novo H3K9me3 occurred shortly after the activation of re-constructed embryos, which is consistent with the establishment of H3K9me3 after fertilization. Notably, genomic regions containing minor zygotic gene activation (ZGA) restricted genes and motifs of YY1 and DUX in cloned embryos were compactly packaged into H3K9me3-heterochromatin, which failed to be reprogrammed and activated. Meanwhile, early SCNT embryos showed defective regulation of H3K9me3-mediated heterochromatin on long terminal repeats (LTR) regions. Finally,

we observed obscure lineage-specific H3K9me3 deposition between inner cell mass (ICM) and trophectoderm (TE) in cloned blastocysts, which may affect further lineage commitment post implantation. Importantly, we identified certain transcription factors that may be essential for H3K9me3-mediated heterochromatin organization during differentiation, which are deficient in cloned blastocysts. Compensation of these transcription factors benefited cloned embryo development and improved lineage specificity of ICM and TE.

Funding Source: This work was supported by the National Key R&D Program of China (2018YFC1004000 and 2020YFA0112500) and the National Natural Science Foundation of China (31721003, 32070802, 31701262).

Keywords: SCNT, H3K9me3, heterochromatin

Poster: 912

DOWNREGULATION OF ODD-SKIPPED RELATED 2, A NOVEL REGULATOR OF EPITHELIAL-MESENCHYMAL TRANSITION, ENABLES EFFICIENT SOMATIC CELL REPROGRAMMING

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Somatic cell reprogramming proceeds through a defined set of events to generate induced pluripotent stem cells (iPSCs). The early stage of reprogramming of mouse embryonic fibroblasts (MEFs) is characterized by rapid cell proliferation and morphological changes, which are underpinned in part by downregulation of mesenchyme-associated genes. However, the functional importance of their downregulation for reprogramming remains poorly defined. We have screened transcriptional regulators that are downregulated immediately upon reprogramming and found that odd-skipped related 2 (Osr2) is downregulated to permit progression through mesenchymal-epithelial transition (MET) for efficient reprogramming. In an epithelial-mesenchymal transition (EMT) model cell line, we show that Osr2 induces cell proliferation, which is followed subsequently by EMT via the TGF-ß signaling pathway. In addition, Osr2 expression seemed to inhibit an activation of Wnt pathway to impair a progression of the reprogramming. Our results identify Osr2 as a novel regulator of EMT and reveal the mechanistic relevance of its downregulation at the early stage of reprogramming.

Keywords: reprogramming, Osr2, TGF-ß

Poster: 913

QUANTITATIVE DNA METHYLATION ANALYSIS OF BIVALENT DOMAINS ALLOWS SELECTING TUMOR-PRONE HUMAN IPS CELL CLONES.

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Differentially methylated regions (DMRs) have previously been reported between human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) using a small number of cell lines for each cell type. We revisited this issue using 32 independent human pluripotent stem cell (hPSC) lines with sufficient methylome read-depth. During mouse reprogramming, Tet1 demethylates somatic enhancers, which fully exploits the developmental potentials of iPSCs. Coincidentally, we have previously established T-iPSCs, where reprogramming using the Yamanaka factors was aided by TET1. In this study, we compared the CpG-methylation status among T-iPSCs, hiPSCs and hESCs. Overall comparison of the global methylation patterns gave little difference between clones. We next investigated the bivalent domains' methylation patterns, known to regulate essential gene expression for development. Bivalent domains randomly gained hypermethylation in most hiPSCs and hESCs. As these bivalent domains are invariably hypomethylated, we deduced that hPSCs might randomly lose bivalency over their gene regulatory regions, manifesting as differentiation propensity. A T-iPSC clone with the highest differentiation capacities (1T47) had the lowest methylation levels at bivalent domains, closely followed by hESC HUES8. To our surprise, the golden-standard hESC H9 bore relatively heavy methylation levels. We next sought to investigate whether the average hiPSCs share common hypermethylated loci (>25%) in non-bivalent candidate cis-regulatory elements (ENCODE3). We compared methylation levels of the top three hypomethylated hPSC clones with those obtained by averaging nine representative iPSC clones. This comparison depicted 141 DMRs, of which more than 80% overlapped with promoters or enhancers (vs 20% expected). Surprisingly, commonly hypermethylated genes' enhancers in hiPSCs were also frequently methylated in various kinds of cancers. Given the fact that most DMRs among hPSCs were in promoters and enhancers, our findings suggest TET may improve differentiation potency by demethylating them. We may also use these DMRs as an index for avoiding tumorigenic hiPSCs and hESCs with diminished differentiation potentials into target cell types.

Funding Source: This research was supported by AMED under Grant Number JP20bk0104090.

Keywords: DNA methylation, bivalent domains, TET1

Poster: 914

LINEAGE SEGREGATION AND THE PLURIPOTENCY CONTINUUM IN THE RABBIT PRE-IMPLANTATION EMBRYO

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Pluripotency is the ability of a stem cell to give rise to all cell types in mature organisms. The characterization of the early embryo transcriptome is crucial to understand the regulation of pluripotency and its relationship with the extra-embryonic lineages, as well as capturing the different states of pluripotency in vitro. Pluripotent embryonic cells appears during the formation of the blastocyst, when the inner cell mass (ICM) segregates from the trophoblast lineage. Pluripotency is then maintained in the cells of the epiblast during the segregation of the primitive endoderm. The primordial germ cells segregation from the epiblast occurs rapidly after the implantation. Finally,

the epiblast undergo gastrulation, first in the posterior part of the epiblast. During this time frame, embryonic cells go through three pluripotency states. Naïve-state pluripotency characterizes the ICM/epiblast of pre-implantation embryos. Formative pluripotency characterize the epiblast of peri-implantation embryos. Finally, primed pluripotency characterizes the epiblast cells of early post-implantation, gastrulating embryos. In most species, the obtention and study of peri-implantation and post-implantation embryos is difficult, as they are already implanted inside the uterine wall. In rabbits however, embryos implant themselves after the onset of the gastrulation. This enlarged developmental window of easy to access embryos makes rabbits a valuable model to study the pluripotency continuum. Using single-cell 10x Chromium RNA-sequencing of rabbit embryos, we analysed the separation points of the embryonic lineages, as well as the expression dynamic of pluripotency regulators. We completed this analysis by using immuno-staining of specific markers. From those data, we identified novel molecular markers that characterize the naïve, formative and primed state of pluripotency in rabbits. Those discoveries could be used to capture naïve- and formative-state pluripotency in rabbit PSC.

Funding Source: ANR Labex "REVIVE" (ANR-10-LABX-73), LabEx "DEVweCAN" (ANR-10-LABX-0061) of the University of Lyon within the program 'Investissements d'Avenir' (ANR-11-IDEX-0007), INRAE Animal Physiology and Livestock Systems Division.

Keywords: pluripotency continuum, pre-implantation embryonic development, lineage segregation

Poster: 915

SPEN IS REQUIRED FOR XIST UPREGULATION DURING INITIATION OF X CHROMOSOME INACTIVATION

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To ensure X-linked gene dosage compensation between females (XX) and males (XY), one X chromosome undergoes X chromosome inactivation (XCI) in female cells. This process is tightly regulated throughout development by many different factors, amongst them, 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, via recruitment of repressor proteins SMRT and HDAC3, leading to histone deacetylation and PolII exclusion. Our results show that Spen null female mouse embryonic stem cells (ESCs) are defective in Xist upregulation upon differentiation. We find that Xist-mediated SPEN recruitment to the Xi chromosome happens very early in XCI, and that SPEN mediated silencing of the Tsix promoter is required for Xist upregulation. Accordingly, failed Xist upregulation in Spen-/- ESCs can be rescued by concomitant removal of Tsix. These findings indicate that SPEN is not only required for the establishment of the Xi, but is also crucial in the initiation of the XCI process.

Keywords: X chromosome inactivation, Gene transcription regulation, Long non-coding RNA

Poster: 916

UNRAVELLING THE ROLE OF PRC2 IN HUMAN PLURIPOTENT STEM CELLS

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During early human development, chromatin changes take place as totipotent stem cells differentiate into all different embryonic and extra-embryonic cell lineages that will form a human being. Yet, how chromatin regulators mediate cell fate during the first stages of human embryonic development is unknown. Here, we combined histone post-translational modification profiling, chromatin proteomics and transcriptional analyses to investigate the changes governing human naïve and primed pluripotency. We found several chromatin complexes, including DNMT3L and transcription factors, such as PRDM14, to be significantly enriched in naïve over primed chromatin. In addition, on primed chromatin, we found enrichment of the pluripotency transcription factors LIN28A and L1TD1 and of the chromatin regulators RCOR2 and SMARCA1. We also found that the repressive mark H3K27me3, deposited by the Polycomb Repressive Complex 2 (PRC2), is enriched in naïve over primed chromatin. To further investigate the role of PRC2 in human pluripotency, we inhibited PRC2 and found a switch in the histone modifications landscape in naïve cells. However, these changes were not accompanied by large effects in gene expression or chromatin-associated proteins. Importantly, we found that PRC2 activity restricts trophoblast cell fate induction from the naïve state. We will also present our progress analyzing the transcriptional and cellular function of PRC2 during the naïve to trophoblast conversion. Investigating the chromatin and transcriptional mechanisms regulating the maintenance of human pluripotent states will have major implications towards a more complete understanding of the first stages of a human life.

Funding Source: This work is supported by the FWO, KU Leuven, BBSRC and MRC.

Keywords: human pluripotency, PRC2, trophoblast stem cells

Poster: 917

LARGE SCALE ANALYSIS OF IMPRINTING IN NAIVE HUMAN PLURIPOTENT STEM CELLS REVEALS RECURRENT ABERRATIONS AND A LINK TO FGF SIGNALING

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Naive human pluripotent stem cells (hPSCs) hold great potential for studying molecular processes and cell fate decisions that occur in human pre-implantation embryos. Genomic imprinting is a parent-of-origin dependent monoallelic expression of a subset of genes and is required for normal growth and development. Previous studies showed that the conversion of primed hPSCs into naive pluripotency is accompanied by genome-wide loss of methylation that includes imprinted loci. However, the extent to which the loss of methylation at imprinted loci causes aberrant bi-allelic expression of imprinted genes is still unknown. Here we analyze loss of imprinting (LOI) in a large cohort of both bulk and single cell RNA sequencing samples of naive and primed hPSCs. We show that naive hPSCs exhibit much higher levels of LOI and biallelic expression than their primed counterparts. This imprint loss is not random, but rather shows a clear bias towards certain imprinted regions. Specifically, genes under the control of paternally methylated imprinting control regions (ICR) are more prone to lose imprinting than genes under the control of maternally methylated ICR. Importantly, we show that different protocols used for the primed-to-naive conversion led to different extents of LOI, a difference which is tightly correlated to FGF signaling. This analysis sheds light on the process of LOI that occurs during the conversion to naive pluripotency and highlights the importance of taking these events into consideration when modeling disease and development or when utilizing the cells for therapy.

Keywords: Naive Pluripotent Stem Cells, Epigenetics, Parental Imprinting

Poster: 918

CHARACTERIZATION OF CELLULAR HETEROGENEITY AND AN IMMUNE SUBPOPULATION OF HUMAN MEGAKARYOCYTES

Zhou, Jiaxi[1], Liu, Cuicui[1], Wu, Dan[1], Xia, Meijuan[1], Li, Minmin[1], Shen, Biao[1], Su, Pei[1], Zhu, Xiaofan[1], Wang, Qianfei[2], Gao, Xin[1], Cheng, Tao[1]

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Megakaryocytes (MKs) and their progeny platelets function in a variety of biological processes including coagulation, hemostasis, inflammation, angiogenesis and innate immunity. However, the divergent developmental and cellular landscape of adult MKs remains mysterious. Here, by deriving the single-cell transcriptomic profiling of MKs from human adult bone marrow (BM), we unveiled cellular heterogeneity within MKs and identified a MK subpopulation with high enrichment of immune-associated genes. By performing the dynamic single-cell transcriptomic landscape of human megakaryopoiesis in vitro, we found that the immune signatures of MKs could be traced back to the progenitor stage. Furthermore, we identified two surface markers, CD148 and CD48, for mature MKs with immune characteristics. At the functional level, these CD148+CD48+ MKs can respond rapidly to immune stimuli both in vitro and in vivo, exhibit high-level expression of immune receptors and mediators, and might function as immune-surveillance cells. Our findings uncover the cellular heterogeneity and a novel immune subset of human adult MKs and should greatly facilitate the understanding of the divergent functions of MKs under physiological as well as pathological conditions.

Keywords: Adult MKs, Cellular heterogeneity, CD48

Poster: 920

HUMAN IPSC LINES WITH KNOCKOUT OF DIFFERENT DNMT3A EXONS DO NOT ACQUIRE DE NOVO DNA METHYLATION DURING DIFFERENTIATION

Cypris, Olivia[1], Franzen, Julia[1], Kuo, Chao-Chung[1], Schmitz, Stephani[1], Frobel, Joana[1], Selina, Nüchtern[1], Sontag, Stephanie[2], Zenke, Martin[2], Wagner, Wolfgang[1]

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DNA methylation (DNAm) is a fundamental epigenetic process, which is tightly regulated during cellular differentiation. It is largely unclear how de novo DNA methyltransferases, such as DNMT3A, are guided to specific sites in the genome. DNMT3A has 23 different exons that are combined into 5 alternative transcripts – and these might have different binding preference. So far, only few human pluripotent stem cell lines with knockout of DNMT3A have been described, mostly with non-sense mutations in exon 19. To further investigate the functional relevance of DNMT3A variants, we generated six human induced pluripotent stem cell (iPSC) lines with knockout of exon 2, 19, or 23: exon 2 comprises an N-terminal start codon, whereas exon 19 and 23 contribute to the catalytic domain. CRISPR/Cas9 knockouts were confirmed by PCR, Sanger sequencing, and quantitative RT-PCR. Western blot analysis showed truncated DNMT3A for exon 2-/-, whereas it was completely removed in exon 19-/- and exon 23-/-. All knockouts revealed iPSC-like morphology with trilineage differentiation potential and positive Epi-Pluri-Scores. While in pluripotent state, there were hardly any differences in genome-wide DNAm profiles as compared to wildtype. Subsequently, we analyzed the impact of DNMT3A knockouts on differentiation of iPSCs towards mesenchymal stromal cells (iMSCs): All lines revealed similar fibroblastoid morphology and immunophenotypic changes. Yet, exon 19-/- and exon 23-/- did not gain any de novo methylation during differentiation, except for very few CG dinucleotides (CpG sites). Furthermore, all iPSC lines could be differentiated into hematopoietic progenitor cells (iHPCs) with typical hematopoietic morphology, up-regulation of hematopoietic surface markers, and colony forming unit potential. Again, on epigenetic level there was hardly de novo methylation in exon 19-/- and exon 23-/- iHPCs. Notably, the very few CpGs that still gained DNAm were overlapping in iMSCs and iHPCs and associated with pluripotency related transcription factor binding sites. Taken together, knockout of exon 2, 19,

and 23 of DNMT3A hardly impact on directed differentiation of human iPSCs. However, knockouts of exon 19 and 23 almost entirely disabled de novo DNAm of iMSCs and iHPCs, indicating that this process is largely dependent on DNMT3A activity.

Funding Source: German Research Foundation (WA 1706/8 1, WA 1706/12-1); IZKF within the faculty of Medicine at RWTH Aachen University (O3-3), Deutsche Krebshilfe (TRACK-AML); Federal Ministry of Education and Research (VIP + Epi-Blood-Count)

Keywords: DNMT3A, DNA methylation, epigenetic

Poster: 921

HIPSC GENERATION AND REDIFFERENTIATION INTO HUMAN INDUCED MYOGENIC CELLS IS DEPENDENT ON THE SOMATIC CELL TYPE OF ORIGIN?

Metzler, Eric[1], Escobar, Helena[1], Sunaga-Franze, Daniele Yumi[2], Sauer, Sascha[2], Spuler, Simone[1]

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Human induced pluripotent stem cells (hiPSCs) represent the keystone to unrestricted cell numbers which are necessary for gene correction and repopulation of large organs such as skeletal muscle in genetic muscular dystrophies. hiPSCs have been generated from many different cell types and several protocols have been established to differentiate them into induced myogenic cells (iMCs). However, the biotechnological and therapeutic capabilities of these iMCs remain unclear. In addition, whether the epigenetic memory passed on to the hiPSCs from the somatic cell type they originated from has an influence on their myogenic differentiation capacity has not yet been examined. Myoblasts, regularly obtained from muscle biopsy specimen, and peripheral blood mononuclear cells (PBMCs) represent the most relevant cell types for the generation of autologous hiPSCs for the treatment of muscular disorders. Thus, we generated hiPSCs from primary muscle cells and compared their molecular profiles and differentiation potential to blood-derived hiPSCs from the same donors. Our results show 40 times higher reprogramming efficiencies using myoblasts compared to PBMCs. Further analysis showed 122 significantly differentially regulated transcripts comparing myoblast- and PBMC-derived hiPSCs. We found no difference in the myogenic differentiation potential evaluating the number of myogenic cells after in vitro differentiation into myogenic cells using both cell types. Furthermore, we show the in vivo myofibre regenerative potential for both, myoblast- and PBMC-derived new myogenic cells after transplantation into an immunocompromised mouse model. Notably, we found pronounced differences in the myogenic differentiation efficiency in between donors, clearly showing an influence of the donor's genetic background on the ability of hiPSCs to differentiate into the myogenic lineage in vitro. We conclude that myoblast- and PBMC-derived hiPSCs harbour a comparable myogenic potential while myoblasts are a highly efficient source to generate autologous hiPSCs for patients with muscular dystrophies due to their high reprogramming efficiency.

Keywords: Reprogramming, Myogenic differentiation, Epigenetic memory

Poster: 922

NEURAL DIFFERENTIATION OF MOUSE CEREBELLAR GRANULE CELL PROGENITORS ARE CONTROLED BY JDP2-MEDIATED ANTIOXIDATION

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Jun dimerization protein 2 (JDP2) was mainly expressed in cerebellar progenitor granule cells (PGCs) in the cerebellum. In addition, Jdp2-knock out (KO) mice exhibited 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, we found 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). 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. Moreover, 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 pivotal role of p21Cip1 in controlling oxidative stress and apoptosis of PGCs in the absence of JDP2. This indicated the retarded maturation of Jdp2-KO PGCs to form the altered lobes. We have generated the cerebellar granule stem-like cells using the reprogramming methods and examined their neural differentiation. The differentiation program to neuron in Jdp2-KO PGC-stem like cells was faster than that of WT PGC-stem like cells. The characterization of neuronal cell types and its mechanism to differentiation will be discussed.

Funding Source: Ministry of Science and Technology (MOST109-2314-B-037-119, MOST109-2320-B-037-033); National Health Research Institutes (NHRI-EX109-10720SI); Kaohsiung Medical University (KMU-M106001, KMU-TC108A02, SA10803C).

Keywords: Cerebellum granule cell progenitors, Jdp2, Antioxidation

Poster: 923

THE ROLE OF GALECTIN-3 IN THE FORMATION OF H9-DERIVED NEURAL ROSETTES

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Stem cell therapy harbours a great potential for treating neurodegenerative disorders. However, it is not a commonly used approach due to the complexity of neural stem cell regulatory mechanisms. Galectin-3 is a lectin that acts via interacting with

glycoconjugates, which results in activation of intracellular signalling, cytoskeleton remodelling and intracellular trafficking. Although Galectin-3 is strongly expressed in the subventricular zone neurogenic niche in the brain, its specific functions in neural stem cells remain to be elucidated. Here, I show that Galectin-3 is an important regulator of neural differentiation. Using an in vitro model of human embryonic stem cells (hESCs) line H9-derived neural differentiation, I observed that Galectin-3 is expressed and secreted at early days of neural induction. During that process, Galectin-3 regulates cell polarisation but not the expression of neural markers. It was possible to see that the protein is localised into vesicles, although the specific compartment remains to be elucidated. Finally, I observed that Galectin-3 blockage affected the delivery of the Hippo pathway's main effector, the co-transcriptional factor YAP, to the apical region of neural stem cells. Collectively, these results show that Galectin-3 regulates neural differentiation via a mechanism that impacts on establishment of apical/basal polarity and YAP localisation. These findings help to clarify how neurodevelopment is regulated by offering a new set of integrated molecular mechanisms involved with neural differentiation.

Funding Source: This study was funded by a Medical Sciences Graduate School Studentship of the University of Oxford, composed of Cancer Research UK (CRUK) and Clarendon Studentships.

Keywords: galectin-3, neural rosettes, YAP

Poster: 924

SOX2 REGULATORY REGION 2 (SRR2) DELETION BY CRISPR/CAS9 REDUCES ONCOGENIC ACTIVITY IN GLIOBLASTOMA

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SOX2 is a transcription factor associated with stem cell activity in several tissues. SOX2 expression is elevated in a large proportion of cancers, such as glioblastoma (GB), the most common malignant primary brain tumor in adults. SOX2 acts as a relevant driver of GB progression and high levels are associated with the population of tumor initiating cells and poor patient outcome. Transcriptional regulation of SOX2 is complex and not fully understood. The SOX2 regulatory region 2 (SRR2) is located downstream of the SOX2 coding region and mediates SOX2 expression by the association of p21CIP1 and p27KIP1 to SRR2. In this study we dissected the role of SRR2 on SOX2 activity in GB. For this, we deleted SRR2 (SRR2del) by CRISPR/Cas9 technology in GB cells. We report that the deletion of SRR2 in GB cells leads to a reduction of SOX2 expression, which was accompanied with an impairment of cell growth and proliferation as well as with a reduction of self-renewal capacity in vitro. These data reveal that SRR2 is required for SOX2 expression and that its deletion results in impaired tumorigenic capacity of GB cells. These effects correlated with an increased expression of p21CIP1 and p27KIP1 together with high levels of p53 in SRR2del cells. On the contrary, SRR2del cells presented decreased levels of stem cell markers, supporting the idea that SRR2 is necessary for the SOX2-mediated regulation of stemness pathways. Furthermore, ectopic overexpression of SOX2 in SRR2del cells restored SOX2 expression as well as proliferation and stem cell properties, indicating that the expression and the oncogenic activity of SOX2 is regulated by SRR2. Additionally, our xenograft models revealed that the lack of SRR2 impairs tumor initiation and growth in vivo. Tumors derived from SRR2del cells were significantly smaller compared to controls and exhibit low levels of SOX2, Ki67 and BMI1 but high levels of p21CIP1 and p27KIP1. Our data confirm that the SRR2 regulates SOX2 expression and reveal that SRR2 deletion reduces SOX2 levels and halts malignant activity driven by SOX2 in GB.

Keywords: SOX2, Glioblastoma, Stem cells

Poster: 925

NGN2 INDUCES DIVERSE NEURONAL LINEAGES FROM HUMAN PLURIPOTENCY

Lin, Hsiu-Chuan[1], He, Zhisong[2], Ebert, Sebastian[2], Schörnig, Maria[3], Santel, Malgorzata[2], Weigert, Anne[3], Hevers, Wulf[3], Kasri, Nael N.[4], Taverna, Elena[3], Camp, Gray[1], Treutlein, Barbara[2]

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Human neurons engineered from induced pluripotent stem cells (iPSCs) through Neurogenin 2 (Ngn2) overexpression are widely used to study the mechanisms controlling neuronal differentiation and to model human neurodevelopmental and neurological diseases. However, the differentiation paths and heterogeneity of neurons that are produced through this cell fate programming method have not been fully explored, thus limiting the ability to interpret the results gained from averaging across an ensemble of cells. Here we used single-cell transcriptomics to dissect the cell states that emerge during Ngn2 overexpression across a time course from pluripotency to neuron functional maturation. We find that there is substantial molecular heterogeneity in the neuron types that are generated, with at least two populations that express genes associated with neurons of the peripheral nervous system. Neuron heterogeneity is observed across multiple iPSC clones and lines from different individuals. We find that neuron fate acquisition is sensitive to Ngn2 expression level and the duration of Ngn2 forced expression. Our data reveals that Ngn2 dosage can regulate neuron fate acquisition, and that Ngn2-iN heterogeneity can confound results that are sensitive to neuron type.

Funding Source: HCL is supported by the Human Frontier Science Program (LT000399/2020-L)

Keywords: Ngn2, iNeuron, scRNA-seq

00:00 - 1:00 EDT

POSTER SESSION 9

MODELING DEVELOPMENT AND DISEASE

Poster: 657

FEEDER-FREE CULTURE OF PIG EMBRYONIC STEM CELLS AND THEIR MYOGENIC POTENTIAL

Lee, Chang-Kyu, Choi, Kwang-Hwan, Lee, Dong-Kyung, Kim, Seung-Hun, Oh, Jong-Nam, Lee, Mingyun, Jeong, Jinsol, Choe, Gyungcheol, Lee, Chang-Kyu

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Pig embryonic stem cells (ESCs) are considered a promising resource for preclinical research on human therapies and cellular agricultural biotechnology such as cultured meat. Previously, we developed the chemically-defined culture media that are capable of maintaining authentic pig ESCs in vitro. However, feeder cells made of mouse embryonic fibroblasts are still used and cause culture inconsistencies for in vitro culture of pig ESCs. Here, we validated whether chemically-defined media can maintain pig ESCs without feeder cells during an extended period. Pig ESC lines cultured with feeder cells were transferred into Matrigel-coated plate and were maintained for multiple passages. In the absence of feeder cells, pig ESCs were stably maintained over about 10 passages and were capable of forming teratomas that contained three germ layers. Immunostaining showed that the stem cells expressed pluripotency markers including OCT4, SOX2, NANOG, SSEA1, and SSEA4. The pig ESCs cultured without feeder cells have similar transcriptome to the stem cells cultured with feeder cells as revealed by RNA-seq. Furthermore, the pig stem cells have the ability of direct differentiation into myoblasts through the paraxial mesoderm by modulation of WNT and BMP pathways. In conclusion, we found that chemically-defined media can support pig pluripotency in the absence of feeder cells as well. Also, pig muscle stem cells could be derived from ESCs. Our findings will facilitate both the development of large animal models for human stem cell therapy and the agricultural applications of pluripotent stem cells from domestic animals.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT; 20012411), and the National Research Foundation of Korea (NRF) grant (2021R1A2C4001837).

Keywords: porcine embryonic stem cells, feeder-free culture, myogenesis

Poster: 935

ENGINEERING CARDIOGENESIS

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Advances in bioengineering now enable complex tissue assembly and simulations of morphogenic events in vitro, creating robust modelling platforms of human development for the first time. The embryonic myocardium of the primary heart tube undergoes rapid and asymmetrical architectural transformation initiated by polarising cues from the adjacent endocardium and the cardiac-specific extracellular matrix (ECM), termed cardiac jelly. The signals that deliver a polarising instruction in a three-dimensional system can now be emulated in a controllable laboratory format using established technologies of pluripotent stem cell-derived cardiovascular tissue engineering and emerging ones of intravital bioprinting. Consequentially, reverse engineering the human embryonic heart represents a versatile method for uncovering human data in a field populated almost exclusively by animal studies. Using human induced pluripotent stem cell (hiPSC)-derived cardiac progenitor cells (CVPs) seeded onto multiphoton-bioprinted cardiac jelly-mimicking scaffolds we have engineered the embryonic heart tube with a view to explore how the myocardium becomes polarised in utero. Bioprinted scaffolds are designed with geometries, biochemical composition and biophysical properties of embryonic heart ECM, generating a platform upon which hiPSC-CVP on-scaffold differentiation to cardiomyocytes closely mimics heart tube formation; namely transmural matrix contact and exogenous simulations of endocardial-to-myocardial signalling in a highly biomimetic tissue model. Motile CVPs highly expressive of MESP1 are seeded onto bioprinted scaffolds to differentiate functionalised contractile tubular tissues, maintaining lumens that occlude with contractions and displaying organised sarcomeric alignment. Engineered Cardiac Tubes host spontaneous hyaluronan (HA) and versican (VCAN) secretions reminiscent of cardiogenesis, and exhibit a morphogenic priming in the form of proliferation and trabecular fate bias in response to the endocardial signalling mitogen Neuregulin-1β (NRG1β). We anticipate Engineered Cardiac Tubes to become a versatile format for uncovering human data of cardiogenesis, revealing new insights into mechanisms underlying human trabeculogenesis in a modelled system for the first time.

Funding Source: This work is funded by the British Heart Foundation 4-Year PhD Studentship in Cardiovascular Biomedicine, and the National Institute for Health Research (NIHR); the University College London Hospitals Biomedical Research Division.

Keywords: cardiogenesis, tissue engineering, bioprinting

Poster: 936

MASSIVE EXPANSION OF HIPSC-DERIVED CARDIOMYOCYTES RESULTING IN EFFICIENT BIOBANKING AND FUNCTIONAL 3D TISSUE ANALYSIS OF GENETIC CARDIOMYOPATHIES.

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Since the discovery of human induced pluripotent stem cells (hiPSCs), numerous strategies have been established to efficiently differentiate hiPSCs into cardiomyocytes (hiPSC-CMs) for their usage in downstream applications such as disease modelling, drug-screens and cell therapy. For all of these applications, robust generation of large quantities of homogenous hiPSC-CMs from various donors, yet, remains a significant hurdle. Subsequent expansion of hiPSC-CM cultures is generally modest (<10 fold). Here, we describe a cost-effective strategy for massive expansion (> 250 fold) of high-purity hiPSC-CMs relying on two aspects: 1.) Inhibition of cell-cell contact via low-density seeding and serial passaging in culture flask-format. 2.) Small molecular glycogen synthase kinase-3 beta (GSK3) inhibition with CHIR99021 (CHIR). Moreover, our technique allows for bio-banking of iPSC-CMs and subsequent thawing and expansion with similar efficiency to non-cryopreserved cells. Using this strategy, it is possible to produce 1 billion cardiomyocytes within 3-4 weeks starting with one differentiation batch (1 single 6-well with purity of 89±11%) of day 11 hiPSC-CMs, without the need for cell sorting or selection. Remarkably, within hiPSCs differentiated with directed differentiation strategies, GSK3 inhibition selectively promotes cell-division of the CM fraction and therefore has purifying effects in (impure) differentiation batches. Expanding hiPSC-CMs retain a fully functional and immature phenotype, and upon withdrawal of CHIR and loss of cell-cell contact inhibition, the cardiomyocytes remain the capacity to terminally differentiate and mature. Previously expanded hiPSC-CMs are fully functional and can be utilized for advanced tissue engineering applications such as fibrin-based engineered heart tissues (EHTs). Patient-specific hiPSC-CMs harbouring a PLNR14del mutation showed a reduced force phenotype (0.137 ± 0.012) vs healthy control (0.229 ± 0.030) and isogenic control (0.224 ± 0.008) in previously expanded CMs. Altogether, this method forms a well-controlled platform for upscaling hiPSC-CM production as is required for 3-dimensionale cardiac disease modelling, large drug screenings and regenerative therapy applications.

Funding Source: UMC Utrecht Clinical Fellowship, Netherlands Heart Institute Fellowship, CVON-Dosis young talent grant, Foundation Leducq (Cure-PLaN), PLN foundation.

Keywords: hiPSC-CMs, Wnt signaling, cardiomyocyte proliferation

Poster: 937

FOLLISTATIN-LIKE 1 PROMOTES CARDIOMYOCYTE PROLIFERATION AND PROTECTION IN A HUMAN IN VITRO CARDIAC DAMAGE MODEL

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The human heart has limited regenerative capacity and cannot repair itself after injury. Therefore, patients often progress to heart failure after ischemic injury despite a decrease in direct mortality by reperfusion therapies. Ischemic injury does increase the number of mitotic cardiomyocytes (CM) and in neonatal and amphibian hearts in sufficient numbers to lead to complete cardiac regeneration. Factors able to increase CM mitosis in the adult mammalian heart have also been identified in animal models but remain ineffective in clinical translation. Glycoprotein Follistatin-like 1 (FSTL1) has been shown to increase CM proliferation and decrease CM apoptosis in animal models depending on its glycosylation state. To explore its therapeutic potential, we assessed the effects of human FSTL1 in an in vitro model of cardiac ischemic injury using human induced pluripotent stem cell (hiPSC) derived CMs. The effect of CM metabolic maturation on susceptibility to ischemia was determined. Cells were treated with non-glycosylated hFSTL1 (bac-hFSTL1) or glycosylated hFSTL1 (mam-hFSTL1) post-ischemia to analyse glycosylation dependent functionality of FSTL1. Only metabolically matured CMs were sensitive to oxygen and nutrient deprivation (TUNEL+ CMs: 7,5% ± 1,3% [control]; 42,2% ± 11,0%, p<0,001 [24 hours 1% O2]) while immature CMs did not show decreased survival up to 24 hours of ischemia. Bac-hFSTL1 increased KI67 (11,5% ± 3,1% vs 8,4% ± 1,6%, p<0,05), pH3 (4,1% ± 3,0% vs 1,1% ± 0,79%, p<0,01), and AURORA B (3,8% ± 2,0% vs 1,8% ± 0,69%, p<0,05) expression compared to untreated and mam-hFSTL1 treated cells. Furthermore, bac-hFSTL1 increased EdU incorporation (6,2% ± 1,6% [0 ng/mL]; 19,0% ± 1,9% [50ng/mL], p<0,001). Both hFSTL1 versions decreased the number of TUNEL+ CMs post-ischemia (37,6% ± 3,5% [control]; 22,7% ± 1,2% [bac-hFSTL1); 24,9% ± 1,5% [mam-hFSTL1), p<0,01). Bac-hFSTL1 moderately increased RNA expression of cell cycle genes (KI67, CYCLIN D2) and decreased the BAX/BCL2 ratio indicative of decreased apoptosis. Taken together, we have shown bac-hFSTL1 can induce proliferation and protect from ischemia-induced apoptosis in human CMs. It would be of interest to further investigate the underlying mechanisms of these promising effects of FSTL1 in human cardiac cells, before advancing to translational studies.

Funding Source: Marijn Peters is supported by a Netherlands Cardiovascular Research Initiative (CVON) grant (REMAIN 2014B027).

Keywords: Cardiomyocyte proliferation, Cardiac regeneration, Follistatin-like 1

Poster: 938

A HUMAN IPS-MODEL FOR VASCULAR CALCIFICATION REVEALS 9P21 INFLUENCE ON CALCIFICATION ONSET

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Using genome wide association studies (GWAS) our group contributed to the identification of 175 coronary artery disease (CAD) loci where the 9p21 locus showed a strong association which was also reported to be associated with coronary artery calcification (CAC). We established an iPSC-based model for studying vascular calcification and here present the analyzes of the effect of the 9p21 locus on calcification in vitro. Induced-pluripotent stem cells (iPSCs) carrying a deletion of the 60 Kb CAD risk region within the 9p21 locus were differentiated into calcifying vascular smooth muscle cells (VSMCs). Human iPSCs as well as VSMCs were characterized regarding their 9p21 genotype. Four genotypes the non-CAD risk wildtype (WT), CAD-risk (R), risk-KO (R-KO) and non-risk-KO (NR-KO) were used. Calcification deposits were analyzed using calcification specific stainings Alizarin-Red-S and Calcein, and quantified using the Calcein staining and a python script. We detected

increased calcium deposits in the Risk (R) wildtype (WT) as well as R and non-risk (NR) KO genotype. This implicates that the loss of or genetic variants in the 9p21 CAD risk locus increases the risk for vascular calcification. Further, a screening of cell cycle associated genes identified changes in cell cycle regulation between the genotypes, as well as between uncalcified and calcified VSMCs.

Keywords: Coronary artery calcification, induced pluripotent stem cells, iPS-derived vascular smooth muscle cells

Poster: 939

MODELLING THE HUMAN DIABETIC HEART, USING ENGINEERED HEART TISSUE, FOR IN VITRO TESTING OF CARDIOPROTECTIVE DRUGS

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Diabetes is a global epidemic, with cardiovascular disease the leading cause of death in diabetic patients. There is a pressing need for an in vitro model 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. Despite numerous studies using in-vitro and animal models, current preclinical models do not recapitulate the adult human heart. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have potential as a predictive tool but are hindered by their immature phenotype. 3D culture of hiPSC-CMs as engineered heart tissue (EHT) is the most advanced approach to mature the cells. Here, we show for the first time how to modulate the media to mature cells in the EHT and cause them to become insulin resistant. Culturing EHT in a media containing oleic acid resulted in the maturation of sarcomere actinin structure, electrophysiological properties, gene expression, and a metabolic switch to fatty acid oxidation, recapitulating more mature cardiomyocytes. We developed a diabetic medium by modulating levels of glucose, insulin, and palmitic acid. We observed an increase in fatty acid metabolism and a blunted insulin response of EHT cultured in this media, indicating that the hiPSC-CMs had become insulin resistant. We subjected the insulin-resistant 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. Finally, we treated IR-EHT with Molidustat (BAY85-3934), a prolyl hydroxylase (PHD) inhibitor, to rescue the impaired response to hypoxia. We found that Molidustat can stabilize Hypoxia-Inducible Factor 1-α signaling in hiPSC-CMs and increase glycolysis, thereby validating its potential 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 predictive tool 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: engineered heart tissue, type 2 diabetes, insulin resistance

Poster: 940

MODELLING LMNA-CARDIOMYOPATHY WITH PATIENT-SPECIFIC HUMAN IPSC-DERIVED ENGINEERED HEART TISSUE DEMONSTRATE A STRUCTURAL AND MECHANICAL COUPLING DEFECT

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Mutations in the LMNA gene encoding for lamin A/C belong to the established causes of inherited dilated cardiomyopathy (DCM) and are associated with poor prognosis. Lamin A/C is an essential structural protein, located in the nuclear envelope and is therefore pivotal for a range of cellular functions. The mechanisms leading from the mutations to DCM are incompletely understood. Recent studies underpinned (i) a role of haploinsufficiency (ii) a dominant negative, toxic effect or (iii) structural and genetic abnormalities. A skin biopsy of a laminopathy patient heterozygous for the mutation p.H222P was taken and reprogrammed to induced pluripotent stem cells (hiPSC; H222P). An isogenic control cell line (H222PRep) and a cell line homozygous for a truncating mutation (E223X) were generated with CRISPR/Cas9 gene editing. HiPSCs of all lines were differentiated into cardiomyocytes (CM) with an efficiency ranging from 75% to 98% and subsequently casted into engineered heart tissues (EHTs). EHT force was followed over several weeks by video optical recording. Upon mechanical stress interventions (acute increase in afterload), fractional shortening (FS) dropped more in H222P EHTs (mean 70.1 % FS) than in H222PRep (mean 82.8 % FS) when normalized to its own baseline (14-21 EHTs/ 4 batches). RNA sequencing of EHTs under normal conditions showed already upregulation of genes involved in extracellular matrix remodelling, fibrosis and mechanotransduction (3/1). E223X EHTs showed absence of full-length lamin A/C by Western blot, which was accompanied by a tendency for reduced remodelling capacity in EHT format, a drastic drop in force (to 39.7% of maximum mean force) after 4 weeks of development (n=36/5 batches) and a marked reduction of cell integrity compared to H222PRep remaining at 97.5% (n= 28/3). Taken together, hallmarks of LMNA-DCM such as low force generation and increased fibrotic signalling could be recapitulated in a human 3D in vitro model, particularly under mechanical stress intervention. Identifying a phenotype in the model is the first important step to determine disease mechanisms and test novel therapies.

Keywords: Laminopathy, DCM, Mechanical coupling

Poster: 941

HOMOZYGOUS ALPHA-ACTININ-2 MUTATION RESULTS IN A PRONOUNCED CARDIOMYOPATHIC PHENOTYPE AND PROTEIN AGGREGATION IN HIPSC-DERIVED CARDIOMYOCYTES

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Hypertrophic cardiomyopathy (HCM) is the most prevalent inherited cardiomyopathy and mainly caused by mutations in genes encoding cardiac sarcomere proteins. Recently, we recapitulated the pathophysiology of a missense mutation in ACTN2, encoding the sarcomeric anchor protein α-actinin 2, in human induced-pluripotent stem cell derived-cardiomyocytes (hiPSC-CMs). Next to an altered protein function, accumulation of toxic proteins (=proteotoxicity) might be crucial in HCM. Our group - amongst others - showed alterations of the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) in HCM. This study investigated whether ACTN2 mutations affect the proteolytic machinery. Thus, hiPSC-CMs from an index patient with the heterozygous ACTN2 mutation (c.740C>T; ACTN2het), the isogenic control (ACTN2ic) and homozygous mutant (ACTN2hom) cell lines were cultured in 2D and as 3D-engineered heart tissues (EHTs). ACTN2hom EHTs presented a pronounced cardiomyopathic phenotype characterized by lower force and shorter relaxation than ACTN2ic and ACTN2het EHTs. Immunofluorescence analysis revealed higher cell area, α-actinin 2 aggregates, poorly formed sarcomeres, and a marked accumulation of the ALP shuttle protein p62 in ACTN2hom CMs. Immunoblotting displayed lower levels of α-actinin 2 and accumulation of p62 in ACTN2hom, but unchanged levels of the ALP marker LC3-II (microtubule-associated proteins 1A/1B light chain 3B) in all groups. Inhibition of the UPS induced a marked accumulation of p62 and poly-ubiquitinated proteins, but not of α-actinin 2 in all cell lines. A higher autophagic flux was detected by immunoblotting and immunofluorescence analysis with an mTag-RFP-Wasabi-LC3 reporter construct in ACTN2hom/het than in ACTN2ic CMs. Proteomic but not RNA-seq analysis revealed markedly lower levels of various sarcomeric proteins, including α-actinin 2 in ACTN2hom but not ACTN2het and ACTN2ic CMs and EHTs, indicating an aberrant regulation of key cellular pathways, such as protein degradation. In conclusion, ACTN2hom might represent a late stage of HCM but in both, ACTN2het and ACTN2hom, the ALP was activated, likely to compensate the marked accumulation of p62. Hence, this study proposes a vital role and thus a putative therapeutic target of the ALP during the pathogenesis of HCM.

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Keywords: Hypertrophic cardiomyopathy, α-actinin 2, Proteotoxicity

Poster: 942

HIPSC-DERIVED CARDIOMYOCYTES FROM PATIENTS WITH INFANTILE MITOCHONDRIAL CARDIOMYOPATHY SHOW DIMINISHED MITOCHONDRIAL CAPACITY AND DISRUPTED CALCIUM HOMEOSTASIS

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Infantile cardiomyopathy is a rare disease associated with mitochondrial dysfunction. Patients often die shortly after birth, making patient material for reprogramming rare and studying the disease a challenge. We derived human induced pluripotent stem cells (hiPSCs) from a patient diagnosed with Combined Oxidative Phosphorylation Deficiency 8, a rare autosomal recessive mitochondrial disorder caused by three compound mutations in the nuclear-encoded mt-Alanine-tRNA synthetase 2 gene (AARS2). We used CRISPR/cas9 to create an isogenic line by repairing the c.2872 C>T mutation in the mother's allele, leaving the c.1774 C>T and 2188 G>A mutations. In the patient hiPSC-derived cardiomyocytes (hiPSC-CMs), we found lower rates of oxidative phosphorylation (OXPHOS) and glycolysis but a 30-60% increase in mitochondrial copy number. Mitochondrial protein expression of the AARS2 protein and the OXPHOS complex 2 peptide MTCO1 decreased, while the other OXPHOS complexes remained unchanged. RNA-sequencing found altered KEGG pathways such as hypertrophic cardiomyopathy and dilated cardiomyopathy, caused by altered expression of myofibers and calcium channels. Additionally, metabolic pathways regulating OXPHOS, glycolysis, but also multiple metabolic synthesis pathways were affected. To consolidate these observations, we measured intracellular calcium dynamics. The patient hiPSC-CMs revealed a slower beat rate and altered calcium dynamics, with a more extended peak- and decay-time and decreased calcium release flux. We also observed arrhythmia with short bursts of activity, where the calcium transient curve failed to return to baseline, followed by prolonged periods of inactivity. Finally, we measured contraction under pacing conditions and found that the patient hiPSC-CMs failed to follow pacing frequencies higher than 2 Hz, showing arrhythmic behaviour in their contractile properties. In sum, we showed that hiPSC-CMs carrying mutations in AARS2 had a lower metabolic capacity, dysfunctional calcium handling and contractile mechanics, therefore capturing part of the pathology observed in patients. However, more studies are needed to fully recapitulate the pathology, including complete ablation of mitochondrial OXPHOS complex 1, 3 and 4.

Keywords: hiPSC-derived cardiomyocytes, Mitochondrial disease, Metabolomics

Poster: 943

SGLT2 INHIBITORS (SGLT2I) AS A POTENTIAL PROTECTIVE STRATEGY FOR CHEMOTHERAPY RELATED CARDIOTOXICITY: HUMAN INDUCED PLURIPOTENT STEM CELLS MODEL

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Sodium-Glucose co-transporter 2 (SGLT2) inhibitors significantly reduce cardiovascular mortality and heart failure hospitalization in patients with established cardiovascular diseases. SGLT2i have been shown to reduce oxidative stress and improve cardiac energetics. In the current study, we assessed the potential protective effect of Empagliflozin, an SGLT2 inhibitor, against doxorubicin cardiac detrimental effects using human iPSCs in-vitro models. To determine whether empagliflozin, can reduce or prevent chemotherapy-related toxicity, we evaluated its effect on hiPSC-Cardiomyocytes following various, temporal simultaneous exposures to doxorubicin in escalating dosages. SGLT2i protective effect was evaluated by assessing programmed cell apoptosis levels and relevant gene expression profile. Furthermore, we assessed the functional effect of empagliflozin on hiPSC-derived CM Engineered Cardiac Tissue (ECT) force generation. Our results demonstrate that co-administration of empagliflozin with doxorubicin significantly lowered the amount of programmed cell apoptosis from 22% without empagliflozin to 5% with empagliflozin ($p<0.0001$). Furthermore, through real time qPCR analysis, doxorubicin treated cells exhibited lower levels of superoxide dismutase (SOD2), while co-treatment with empagliflozin restored the expression to normal level. Consequently, lower ROS levels were detected cells co-treated with empagliflozin compared with groups treated solely with doxorubicin. Doxorubicin treated ECTs demonstrated lower active forces while concomitant exposure to doxorubicin and empagliflozin resulted in partial recovery of the active force generation. Our study demonstrates the potential ability of SGLT2 inhibitors to minimize the detrimental effect of doxorubicin on cardiac tissue. Importantly, our study demonstrates the applicability of hiPSC-based platforms for identifying cardioprotective drug for the field of cardiac oncology.

Keywords: Cardio-oncology, SGLT2 inhibitors, Doxorubicin

Poster: 944

SYNERGISTIC WNT PATHWAY AND CHROMATIN MODULATION STABILIZES CARDIAC PROGENITORS FROM HPSC IN VITRO, AND ZEBRAFISH HEART DEVELOPMENT IN VIVO

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The in vitro culture of human cardiac progenitor cells remains a major challenge in biomedicine. Cardiovascular progenitor cells may represent a favourable alternative for cell-based heart repair and provide a valuable model to investigate heart development. Histone Acetyl Transferases (HATs) are regulators of gene transcription, thereby defining progress of cell differentiation at specific developmental stages. In this study we focused on the role of the β-catenin binding HATs CBP and p300 during the differentiation of human pluripotent stem cells (hPSCs)-derived cardiac progenitors. We show that chromatin remodeling combined with WNT pathway modulation by chemical inhibitors (IQ-1 and CHIR, respectively) synergistically enables stabilization of human cardiac progenitors (SCPs), in a process that we denominate “transcriptional uncoupling”. SCPs are characterized by ISL1+/KI-67+ expression in our study; these progenitors are maintained in a quiescent state in the presence of the chemical inhibitors. Upon compound removal, cell autonomous NKX2-5 upregulation hallmarks the “recoupling” to the cardiomyogenic program in SCPs, whilst directed differentiation also enables the induction of endothelial cells and smooth muscle cells, confirming SCPs expected multi lineage potential. ChIP-sequencing together with RNA sequencing showed specific enrichment of core genes involved in early heart development, suggesting that the “uncoupling” process does not seem to interfere with lineage commitment per se. We consequently show that IQ-1 treatment also retains ISL1+ cardiac progenitors in vivo in a dose and stage specific manner during zebrafish heart development, suggesting the existence of a mechanistically conserved switch that holds cardiac progenitors’ differentiation, and proliferation potential. Due to its chemically defined and reversible nature, our approach provides an unprecedented opportunity to dissect the key mechanisms in cardiac progenitor cell biology, also providing new options for human heart regeneration.

Keywords: Histone Acetyl Transferases (HATs), p300, Maintenance of human cardiac progenitors, Transcriptional uncoupling

Poster: 945

HUMAN HEART-FORMING ORGANOIDS RECAPITULATE EARLY HEART AND FOREGUT DEVELOPMENT

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In response to proper stimuli, human pluripotent stem cells (hPSCs) self-organize into three-dimensional structures called "organoids" resembling embryo-like tissue patterns in vitro. Although organoid models of early tissue development have been produced for the intestine, brain, kidney and other organs, similar approaches for the heart have been lacking. Following our recently published strategy, we here direct hPSC differentiation into complex, highly structured "heart-forming organoids" (HFOs). HFOs are composed of a myocardial layer lined by endocardial-like cells and surrounded by septum-transversum-like anlagen; they further contain spatially and molecularly distinct anterior versus posterior foregut endoderm tissues and a vascular network. The architecture of HFOs closely resembles aspects of early native heart anlagen prior to heart tube formation, which is known to require an interplay with foregut endoderm development. We reveal the utility of HFOs to study genetic defects in vitro by demonstrating that NKX2.5 knock-out HFOs show a phenotype reminiscent of cardiac malformations previously observed in transgenic mice, including decreased cardiomyocyte adhesion and hypertrophy. In a novel approach, we modulate endothelial cell and cardiomyocyte induction in HFOs in response to time-dependent treatment with vascular endothelial growth factor (VEGF) or thalidomide. By qualitative and quantitative assessment of morphological changes and lineage composition in HFOs, we demonstrate their applicability as a platform for teratogenicity assessment. Together, an advanced model of early human cardiogenesis and vasculogenesis is presented, opening new perspectives to study mechanisms of human heart development, genetic disorders, and consequences of drug treatment in a dish.

Keywords: Organoid, Heart, Vascularization

Poster: 946

A MULTI OMICS ANALYSIS OF A HUMAN STEM CELL BASED IN VITRO MODEL OF CARDIAC HYPERTROPHY

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Cardiac hypertrophy is an important and independent risk factor for the development of cardiac myopathy that may lead to heart failure. Cardiac hypertrophy manifests as an enlargement of the individual cardiomyocytes, which impairs the function of the heart. The only way to cure an end-stage cardiac myopathy is by heart transplantation, a possibility limited due to lack of donor hearts. Therefore, early diagnosis of cardiac hypertrophy is of outmost importance in order to initiate interventions that may prevent further progression of the disease. The mechanisms underlying the development of cardiac hypertrophy are not well understood. To increase the knowledge about mechanisms and regulatory pathways involved in the progression of cardiac hypertrophy we have developed a human induced pluripotent stem cell (hiPSC)-based in vitro model of cardiac hypertrophy and performed extensive characterization of the model using multi-omics analyses. In a series of experiments, hiPSC-derived cardiomyocytes were stimulated with Endothelin-1 for 8, 24, 48 and 72 hours (h) and their transcriptome, proteome and secretome were analyzed thoroughly. The transcriptomic data show many enriched canonical pathways related to cardiac hypertrophy already at the earliest time point, e.g., cardiac hypertrophy signaling, actin cytoskeleton signaling and PI3K/AKT signaling. Cluster analysis of the differentially expressed genes showed that there are numerous clusters of genes that are misregulated over the time period of 8 to 72h. Interestingly, and in line with the transcriptomics data, the proteomics analysis revealed several enriched pathways important in the hypertrophic response. An integrated transcriptome-proteome-secretome analysis enabled the identification of multimodal biomarkers of high relevance for monitoring early cardiac hypertrophy progression. Taken together, the results from this study demonstrate that our in vitro model displays a hypertrophic response on transcriptomic, proteomic and secretomic level. The results also provide novel insight into the underlying mechanisms of cardiac hypertrophy and novel putative early cardiac hypertrophy biomarkers have been identified that will be further validated to assess their clinical relevance.

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Keywords: cardiac hypertrophy, disease model, in vitro model

Poster: 947

MODELING DIFFERENT ASPECTS OF CARDIOTOXICITY UTILIZING HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES: TOWARDS PROTECTIVE THERAPY FOR THE HEART

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Advances in the development of anti-cancer agents have made cancer treatments more effective, but have also increased the risk for cardiotoxic side effects. Cardiotoxicity is one of the main adverse effects of cancer therapy and has primarily been assessed in different animal models which are not predictive for drug-induced cardiotoxicity in humans. Human pluripotent stem cell (hPSC)-derived cardiomyocytes provide a reliable source of human cardiomyocytes and have already proven valuable for cardiotoxicity studies. We utilized high throughput time-lapse imaging of cardiomyocytes differentiated from an NKX2.5eGFP-α-actininmRuby2 hPSC cardiac reporter for assessment of

cell viability and sarcomeric disarray after treatment with Doxorubicin, one of the most effective anti-cancer drugs. Using bioinformatics, we created binary masks of the GFP area to quantify the survival. After 5 days only 20% of cardiomyocytes survived treatment with 1 uM Doxorubicin when compared to the DMSO control group. We also created binary masks of the α-actininmRuby2 signal to quantify disassembly of the sarcomeres and observed that less than 25% of sarcomeres are still present after 5 days. To study different damage mechanisms, we quantified apoptosis with the apoptotic marker Annexin 5, DNA damage marker pH2AX, oxidative stress using Cell Rox, calcium accumulation and expression of cardiac markers after exposure to different concentrations of Doxorubicin. We compared the Doxorubicin uptake into cardiomyocytes and fast dividing hPSCs. Moreover, we evaluated the cardiotoxic effect of Doxorubicin on a functional level by evaluating calcium transients. To more closely resemble the human heart compared to simple 2D monolayer cultures, we have established a more advanced platform for creating 3D engineered heart tissues (EHTs) which allowed us to measure the effect of a cumulative dose of Doxorubicin on cardiac contraction. We quantified absolute force of contraction every 12 h and observed total loss of contraction 72 h after treatment with 5 or 10 uM Doxorubicin. These human stem cell-based assays provide a versatile screening platform for assessment of cardiotoxicity and enabled us to study the cardiotoxic effect on different cardiac subtypes. Ultimately this will lead to a better and safer treatment of patients.

Keywords: Cardiotoxicity, HPSC-cardiomyocytes, Cardiac tissue

Poster: 948

MANIPULATING MICROTUBULE (DE-) TYROSINATION ALTERS CONTRACTILE BEHAVIOR IN HUMAN IPSC-DERIVED ENGINEERED HEART TISSUES

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While the role of the sarcomere in failing cardiomyocytes (CMs) was thoroughly investigated, less attention was paid to the non-sarcomeric cytoskeleton in heart failure (HF). Recently, non-sarcomeric cytoskeletal changes accompanying HF have been unmasked and identified as a potential therapeutic target, especially the α-tubulin tyrosination/detyrosination cycle. Vasohibin 1/2 and small vasohibin binding protein (SVBP) have been recently shown to form the tubulin carboxypeptidase complex, with tubulin tyrosine ligase (TTL) re-adding the C-terminal tyrosine. Increased tubulin detyrosination and microtubule network density were observed in different forms of human and mouse HF, and recent work uncovered a reduction of detyrosination to restore contractility of HF myocytes. The goal of this study was to evaluate the impact of SVBP and TTL deficiency on contractility in a human cellular context. Human SVBP- and TTL-knock-out (KO) induced pluripotent stem cell (iPSC) lines were created by introduction of a kbp-deletion in the respective genomic locus in a control iPSC-line with CRISPR/Cas9. The three iPSC lines were differentiated into CMs and used either in 2D-culture or cast in engineered heart tissues (EHTs). Molecular analysis was performed by RT-qPCR, Western blot, and immunofluorescence imaging. CM differentiation exhibited >90% cardiac troponin T+ cells for all cell lines. RT-qPCR confirmed the deficiency of the respective KO in iPSC-CMs. Further, the detyrosinated a-tubulin level was markedly lower and higher in SVBP-KO and TTL-KO than in control CMs, respectively, both by Western blot and immunofluorescence analysis. Force development of the EHTs over time did not differ between the genotypes on standard posts (0.28 mN/mm). However, when cast on stiffer posts (0.8 mN/mm) inducing afterload enhancement, under 1.5-Hz pacing, force amplitude was lower in TTL-KO EHTs, whereas relaxation was markedly faster in SVBP-KO EHTs. Time to peak force did not differ between the 3 genotypes. These data showed that tubulin tyrosination and detyrosination affect the contractility parameters differently in human EHTs. The TTL-KO model supports previous findings of higher detyrosination in HF and the SVBP-KO model supports the view that targeting detyrosination holds promise to improve diastolic function.

Funding Source: DZHK (Deutsches Zemtrum für Herz-Kreislauf-Forschung e.V.); Leducq Foundation

Keywords: hiPSC-Cardiomyocytes, Microtubules, Detyrosination

Poster: 949

CLOSING THE GAP: A FOCUS GROUP STUDY ON THE ATTITUDES OF DUTCH PROFESSIONALS AND LAY CITIZENS ON THE CREATION AND RESEARCH USE OF STEM CELL-BASED EMBRYO-LIKE STRUCTURES

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Embryo-like structures (ELS), i.e., stem cell-based models that can mimic (stages and/or regions in) early (human) peri-implantation development, may provide powerful tools to study the period during which most human etiologies rise while simultaneously reducing and replacing the use of animals and/or human embryos in research. Scholars in the humanities and social sciences recognize the potential of ELS to secure the benefits of embryo research in a morally less controversial way, but emphasize that public engagement and support will be essential in harvesting this moral advantage. Studies on public attitudes towards synthetic embryology remain nevertheless significantly scarce. To add to this gap in the literature, we set up a qualitative study with a cross-sectional design aimed at exploring the attitudes of Dutch professionals and lay citizens towards the creation and research use of ELS. Data were collected through four semi-structured focus group interviews (N = 33) between August and September 2020, and consisted of one pilot interview, two interviews with lay citizens, and one interview with professionals. Pilot and lay participants were selected based on the sociodemographic characteristics of the Dutch public. Eligibility for inclusion in the interview with professionals was based on relevant experience and affinity with ethical and legal debates on emerging biotechnologies. From our inductive thematic analysis emerged four themes: (1) trust, distrust, and ambivalence; (2) diversity of ELS-conceptualizations; (3) grounds for moral value and moral standing; and (4) conditions for responsible embryo-like research. Of note, whereas questions on the developmental potential of ELS were critical for both the attitudes of professionals and those of lay

citizens, lay citizens were much more concerned that synthetic embryology could fundamentally change human existence for the worse. In particular, lay participants worried that ELS could stimulate a 'makeable human existence' or be insusceptible to regulatory monitoring and control. These findings corroborate the necessity of the ethical standards proposed by the ISSCR for the generation and culture of viable ELS, and may provide a useful starting point for further discussion and analysis of adequate regulatory parameters.

Funding Source: This study is funded by the Dutch Organization for Health Research and Development (ZonMw), the Research School for Oncology and Developmental Biology (GROW) and the Institute for Technology-Inspired Regenerative Medicine (MERLN).

Keywords: Ethics, Public attitudes, Embryo-like structures

Poster: 950

CREATING TO UNDERSTAND: MOUSE EMBRYONIC STEM CELLS SELF-ORGANIZE INTO TRUNK-LIKE STRUCTURES WITH NEURAL TUBE AND SOMITES

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Post-implantation embryogenesis is a highly dynamic process comprising multiple lineage decisions and morphogenetic changes that are inaccessible to deep analysis in vivo. The developmental engineering of embryo-like structures from stem cells in vitro would overcome this impediment. Gastruloids are aggregates of mouse embryonic stem cells that resemble the organized gene expression space of the post-occipital embryo (i.e. an "embryo without a head"), but lack proper morphogenesis, e.g. presomitic mesoderm does not condense into somites and neural cells do not form a neural tube. We discovered that adding a low percentage of the extracellular matrix surrogate Matrigel unleashes embryo-like architecture in gastruloids, resulting in highly organized "trunk-like structures" (TLSs) with a neural tube and somites. Comparative single-cell RNA sequencing analysis confirmed that this process is highly analogous to mouse development and follows the same stepwise gene-regulatory program. Live imaging demonstrated that the segmentation clock, an oscillator involved in somitogenesis in vivo, ticks at an embryo-like pace in TLSs. Tbx6 knockout TLSs developed additional neural tubes mirroring the embryonic mutant phenotype, and chemical modulation could induce excess somite formation. Mechanistically, the addition of Matrigel resulted in the transcriptional up-regulation of important regulators of morphogenesis (incl. integrins, cadherins) and accumulation of the integrin-binding glycoprotein Fibronectin at the structure-matrix interface. The scalable, tractable, and highly accessible nature of the TLS model makes it a complementary in vitro platform for defining the molecular, cellular, and morphogenetic processes that shape the post-implantation embryo, at an unprecedented spatiotemporal resolution.

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Keywords: embryonic organoids, gastruloids, models of embryo development

Poster: 951

INVESTIGATING THE SUSCEPTIBILITY OF HUMAN FETAL TISSUES TO VERTICAL TRANSMISSION OF SARS-COV2

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The SARS-CoV2/COVID-19 pandemic has brought about unprecedented research efforts in attempts to prevent and treat infection across all ages. SARS-CoV2 infection mechanisms are well established, whereby cell entry occurs through ACE2 with co-activation by TMPRSS2 (with additional surface markers reported, such as CD147 and CTSL1). Clinical reports of perinatal infection in the neonate have led to concerns of a potential mechanism for vertical transmission. Determining whether this occurs in utero or from exchange of bodily fluids during labour, is critical for minimising neonatal infection, which is rarely severe, but associated to largely unknown long-term consequences. Infection may trigger miscarriage during the 2nd trimester (as suspected for SARS and MERS coronaviruses) or disrupt fetal development. Various mechanisms of vertical transmission have been proposed: I) direct infection of the syncytiotrophoblast with breach through the syncytial layers II) infection through trafficked maternal cells III) ascending infection through the vaginal tract (most likely in peripartum infection cases). To assess specific tissue susceptibilities, we analysed RNA sequencing data from public repositories. We then validated this at a gene and protein level across a library of human fetal tissues, placenta and amniotic fluid cells from different gestational ages. Our results show that transcription of COVID-19 associated genes differs markedly in fetal subjects across gestation compared to children and adults. With clinically relevant implications, we show that the fetal lung and placenta do not seem to be susceptible to SARS-CoV-2 infection at any gestational age. Surprisingly, our evidence indicates the gastrointestinal tract has the highest expression of ACE2 and TMPRSS2, suggesting it as a potential primary route of infection - possibly compounded by the lack of mucosa or gastric secretions. Furthermore, this expression increases logarithmically with gestational age, to 5-fold adult tissue levels. Through this study we have shed light on the mechanisms of COVID infection and fetal protection. This is essential to refine peripartum clinical practise and improve the recommendations given to pregnant women.

Funding Source: UK Research and Innovation Medical Research Council - Covid19 Rapid-Response call

Keywords: COVID-19, vertical transmission, SARS-CoV2

Poster: 952

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO CHOLANGIOCYTES

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Cholangiocytes are polarized biliary epithelial cells that represent about 3% of liver cells and play a crucial role in liver functions by modifying the bile produced by hepatocytes and draining it to the intestine via the biliary tree. The development of protocols for the differentiation of human induced pluripotent stem cells (hiPSCs) into cholangiocytes (iChols) is crucial because of the limited access to primary human cholangiocytes. Based on our previously published protocol, we further study the potential of hiPSCs to differentiate into iChols. Thanks to the successive addition of growth factors to mimic the main stages of embryonic development, hiPSCs are firstly differentiated into hepatoblasts, the bipotent progenitors of both hepatocytes and cholangiocytes. We then differentiate iChols by using growth hormone, EGF (epidermal growth factor), and interleukin-6 both in 2D in Petri dishes and in 3D in matrigel. The iChols obtained do not express hepatoblast or hepatocyte markers such as alpha-fetoprotein and albumin but express cholangiocyte markers such as SOX9, osteopontin, cytokeratin 7, the bile salt transporter ASBT and the secretin receptor, as well as the tight junction marker zonula occludens-1 (ZO-1). The differentiated cells also express specific proteins important for cholangiocyte function, including CFTR (cystic fibrosis transmembrane conductance regulator). They have a single primary cilium, a sensory organelle that plays an important role in the modulation of the secretory and proliferative functions of cholangiocytes. When cultured in 3D in matrigel, iChols still express cholangiocyte markers and develop and typical apicobasal epithelial polarity. They form functional cysts with luminal space and also polarized bile ducts, which can perform some of the cholangiocyte functions such as the transport of a fluorescent bile salt analog or the specific transport through multidrug resistance protein 1 (MDR1). The use of hiPSCs thus enables the production of differentiated cells that can be used for the in vitro study of the molecular mechanisms of bile duct development and the modeling of cholangiocytic diseases.

Keywords: hiPSCs, Biliary cells, differentiation

Poster: 955

HNF1A P291FSINSC MUTATION IN MONOGENIC DIABETES RESULTS IN DEVELOPMENTAL DEFECTS IN HUMAN IPSC PANCREATIC ORGANOIDS

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Maturity-onset diabetes of the young 3 (MODY3) is the most common subtype of monogenic diabetes and is caused by heterozygous mutations in the transcription factor hepatocyte nuclear factor 1A (HNF1A). The most prevalent HNF1A mutation in MODY3 is the insertion of a cytosine (C) in the poly-cytidine tract of codon 291(p291fsinsC), a frameshift mutation that induces an early stop codon and can result in the expression of a truncated form of HNF1A protein. Current mouse and human models for MODY3 are very limited and do not fully recapitulate all disease phenotypes. Due to a lack of appropriate disease models, the molecular mechanisms underlying the pathobiology of MODY3 due to p291fsinsC mutation have remained elusive. In this study, we established wild-type, MODY3 patient and CRISPR/Cas9 human induced pluripotent stem cells (hiPSCs)-derived progenitor organoids with the p291fsinsC mutation to study the disease mechanism in-vitro. We investigated the impact of the p291fsinsC mutation on the differentiation capacity of hiPSCs into pancreatic progenitors and beta-like cells in organoids. Patient and CRISPR/Cas9-derived hiPSCs showed a severe reduction in PDX1+ and NKX6.1+ pancreatic progenitor formation which paralleled a reduction in beta-cell differentiation compared to wild-type hiPSCs. Mechanistically, we found that the truncated form of HNF1A protein interacts with HNF1B protein, thus impairing HNF1B-dependent gene expression and stalling endocrine development. By inhibiting this interaction, we rescued HNF1B-mediated gene transactivation. Overexpression of HNF1B in the MODY3 patient organoids resulted in a partial rescue of the phenotype by an increase in PDX1+ progenitor cells, indicating that the truncated version of HNF1A protein has a dominant negative effect on both HNF1A and HNF1B. Our study uncovers a novel mechanism of the p291fsinsC mutation during pancreas development in a new human organoid model of MODY3. The p291fsinsC mutation results in a previously undescribed developmental defect that ranges in severity, offering an explanation for the disease heterogeneity observed in MODY3 patients. Moreover, the pancreatic organoid system could be used for investigating developmental defects associated with other mutations recurrent in monogenic or polygenic diabetes.

Funding Source: Wellcome Trust

Keywords: MODY3, CRISPR/Cas9, organoids

Poster: 956

EVOLUTION OF THE MESENCHYMAL COMPONENT OF HUMAN ESC-DERIVED INTESTINAL ORGANOIDS DURING LONG-TERM CULTURE; EFFECT ON PRO-INFLAMMATORY AND FIBROTIC RESPONSES.

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Human Intestinal Organoids (HIOs) are 3D structures, with epithelial crypts that consist of several epithelial cells subtypes and a surrounding mesenchymal layer. Our aim was to develop 3D HIOs from human embryonic stem cells (hESCs) and to examine the evolution of their mesenchymal component during long-term culture. Additionally, we investigated the effect of the pro-inflammatory cytokines IL-1α and TNF-α on the expression of fibrotic and inflammatory mediators in HIOs. The hESC line H1 (Wicell, Madison, Wisconsin, United States) was cultured and differentiated towards HIOs using the STEMdiff™ Intestinal Organoid Kit (StemCell Technologies, Vancouver, Canada). HIOs were characterized by immunofluorescence in all differentiation stages. The evolution of their mesenchymal component was examined by comparing the mRNA expression levels of fibrotic and mesenchymal markers during the culture passages 1-10. Additionally, HIOs from different passages were stimulated with 5ng/ml IL-1α and 50ng/ml TNF-α for 12 hours and the fibrotic and inflammatory mRNA expression was examined. HIOs were successfully developed as they were stained positive for all studied markers. Regarding the evolution of their mesenchymal component, we observed high expression of CD90, collagen type I, type III and fibronectin that was gradually decreased during passages. As for the fibrotic and inflammatory responses from HIOs, we found that the IL-1α and TNF-α stimulation resulted in statistically significant upregulation of the fibrotic factors, fibronectin, collagen type I and type III in culture passages 2 and 4, but had no effect in culture passages 8 and 10. Similarly, IL-1α and TNF-α stimulation led to the statistically significant induction of the inflammatory chemokines CXCL8, CXCL10 and CXCL11 in culture passages 2 and 4, while no effect was observed in culture passages 8 and 10. In conclusion, our findings indicate that HIOs contain a functional mesenchymal component that is gradually diminished during passages, and underline the significance of the mesenchymal cells' fitness in inflammatory and fibrotic responses. Therefore, inflammatory and fibrotic studies employing HIOs should be focused on early passages, while the mesenchymal component is active.

Keywords: human embryonic stem cells, human intestinal organoids, organoid maturation

Poster: 957

SARS-COV-2 INFECTS THE BRAIN VASCULATURE IN COVID-19 PATIENTS WHICH CAN BE MIMICKED WITH AN HIPSC-DERIVED MODEL OF THE BLOOD-BRAIN BARRIER

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Neurological complications are common in patients with COVID-19. Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causal pathogen of COVID-19, has been detected in some patient brains, its ability to enter the brain and impact its function is not well understood. Beside other, the brain vasculature has been proposed as a potential entry point for SARS-CoV-2 and may turn dysfunctional in COVID-19-diseased patients. Here, we investigated the susceptibility of human induced pluripotent stem cell (hiPSC)-derived brain capillary endothelial cells (BCECs), a major cell type of the blood-brain barrier (BBB), to SARS-CoV-2 infection. We found that BCECs could be infected, but the paracellular tightness of the model was not compromised after infection. This entry of SARS-CoV-2 could be pharmacologically prevented by anti-Spike neutralizing antibodies or significantly reduced by anti-ACE2 specific antibodies or small molecule protease inhibitors targeting TMPRSS2. SARS-CoV-2 was found basolateral when the cells were infected from the apical, vessel lumen-facing side, thus suggesting active transcytosis of the virus to the brain compartment. Findings in post mortem CNS tissue of COVID-19 patients present with astrogliosis in close proximity to brain capillaries, supporting the data from the in vitro model. Together, our data provide evidence for SARS-CoV-2 brain entry across the BBB and support the use of hiPSC-derived cell types as a platform to investigate SARS-CoV-2 susceptibility of the central nervous system, mechanisms of virus-induced BBB and brain dysfunction, and treatment strategies.

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Keywords: Blood-brain barrier, SARS-CoV-2, hiPSC disease modeling

Poster: 958

MODELLING OF PRIMARY CILIARY DYSKINESIA USING PATIENT-DERIVED AIRWAY ORGANOIDS

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Patient-derived human organoids can be used to model a variety of diseases. Recently, we described conditions to culture human airway organoids (AOs) directly from healthy individuals and patients. Here, we first optimize differentiation of AOs towards ciliated cells. After differentiation of the AOs towards ciliated cells, these can be studied for weeks. When returned to expansion conditions, the organoids readily resume their growth. We apply this condition to AOs established from nasal inferior turbinate brush samples of patients suffering from primary ciliary dyskinesia (PCD), a pulmonary disease caused by dysfunction of the motile cilia in the airways. Patient-specific differences in ciliary beating were observed which were in agreement with the genetic mutation present in the patients. More detailed organoid ciliary phenotypes could thus be documented relative to the standard diagnostic procedure. This study demonstrates the utility of organoid technology for investigating hereditary airways diseases such as PCD.

Funding Source: This work was supported by the gravitation program CancerGenomiCs.nl from the Netherlands Organisation for Scientific Research.

Keywords: Airway organoids, primary ciliary dyskinesia, Ciliated cell

Poster: 959

MUTATIONAL SIGNATURE IN COLORECTAL CANCER CAUSED BY GENOTOXIC PKS+ E. COLI

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Various species of the intestinal microbiota have been associated with the development of colorectal cancer, but it has not been demonstrated that bacteria have a direct role in the occurrence of oncogenic mutations. Escherichia coli can carry the pathogenicity island pks, which encodes a set of enzymes that synthesize colibactin. This compound is believed to alkylate DNA on adenine residues and induces double-strand breaks in cultured cells. Here we expose human intestinal organoids to genotoxic pks+ E. coli by repeated luminal injection over five months. Whole-genome sequencing of clonal organoids before and after this exposure revealed a distinct mutational signature that was absent from organoids injected with isogenic pks-mutant bacteria. The same mutational signature was detected in a subset of 5,876 human cancer genomes from two independent cohorts, predominantly in colorectal cancer. Our study describes a distinct mutational signature in colorectal cancer and implies that the underlying mutational process results directly from past exposure to bacteria carrying the colibactin-producing pks pathogenicity island.

Keywords: Colorectal cancer, Genotoxic bacteria, Mutational signature

Poster: 960

THE HEDGEHOG PATHWAY MODULATES CELL PLASTICITY BY ACTIVATING AN EMBRYONIC-LIKE PROGRAM IN MOUSE ESOPHAGEAL PROGENITORS IN VIVO

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Metaplasia is defined as the replacement of a fully differentiated cell type by another. There are several classical examples of metaplasia such as intestinal metaplasia of the stomach, squamous metaplasia of the lung and columnar metaplasia of the esophagus also called Barrett's esophagus. The columnar metaplasia of the esophagus is the main risk factor for esophageal adenocarcinoma, but its cellular origin is still a matter of debate. Few studies have shown that cells from the squamo-columnar junction were competent to initiate columnar metaplasia in vivo. Recent data suggest that keratinocytes from the squamo-columnar junction may participate to columnar metaplasia as well. Nonetheless, there is a lack of evidence to demonstrate that esophageal progenitors can be the source of columnar metaplasia. In this study, we show that the Hedgehog pathway is activated in the keratinocytes from the squamo-columnar junction under physiological conditions, and more broadly in squamous epithelial cells upon chronic acid reflux. Using transgenic mouse models, lineage tracing, single cell RNA sequencing, transcriptomic and epigenetic profiling, we found that the activation of the Hedgehog pathway in esophageal cells modifies their squamous differentiation program in vivo. This process involves an initial step of dedifferentiation by reactivating embryonic esophagus specific transcripts and reopening thousands of embryonic esophagus chromatin regions. Moreover, a subset of these cells undergoes a full squamous-to-columnar conversion and starts expressing some intestinal markers. Conditional knockout shows that Sox9 is required for squamous-to-columnar conversion but not for the step of dedifferentiation. These results illustrate the plasticity of esophageal progenitors and provide insights into the mechanisms through which esophageal cells might participate to columnar metaplasia.

Keywords: Cell plasticity, dedifferentiation, transdifferentiation

Poster: 961

THE FUTURE OF PRECLINICAL DISEASE MODELLING: PATIENT-ON-A-CHIP

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In the recent years, microfluidic systems have shown to be a powerful tool for recreating tissue- and organ-like functions by different approaches, providing basis for developing preclinical assays with improved predictive power. Microfluidic culture platforms conjoining human microtissues in a physiological-like arrangement could provide a translational solution. Starting with assay relevant a few organs, Human-on-a-chip platforms aim to combine more than ten organs to mimic the healthy and diseased physiology with patient specific engineered tissues derived from IPSCs to create the Patient-on-a-chip. This allows to develop personalized medicine solutions for diseases and evaluate drug responses with the patient's specific genetic background. In multi-organ-chip (MOC) platform integrated with an on-chip micro-pump, we established thirteen different organ equivalents combined into twelve different co-culture platforms interconnecting two to four organs. The micro-pump ensures stable long-term circulation of the medium through the tissue culture compartments at variable flow rates, adjustable to physiological mechanical stresses of the respective tissues. The circulating medium emulates the physiological relevant crosstalk between tissue models in long-term co-cultures. We show that in MOC we can study Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) of compounds, organ crosstalk, induce and study diseases such as Diabetes type II, lung cancer and their ability to host 3D organoid constructs in a controlled microenvironment with mechanical and electrophysiological stimuli. The new application opportunities as well as important challenges in realizing the full potential of this technology will be addressed to advance the disease modelling field.

Keywords: Organ-on-a-chip, Multi-organ-chip, Patient-on-a-chip

Poster: 962

OPTIMIZING GENOMIC CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) hold an enormous potential in translational research as tools to elucidate disease aetiology and design novel therapies. However, these applications may be compromised by the accumulations of genetic instability leading to chromosomal aberrations, copy number variations and loss of heterozygosity during in vitro hPSC culture and expansion processes. The functional consequences of specific variants are little understood and probably context-dependent. Nonetheless, the acquisition of genetic abnormalities could introduce severe risk factors for the development of reliable disease models as drug discovery platforms and might eventually jeopardize result robustness and clinical utility. As a biorepository and cellular facility, ISENET Biobanking is specialized in cell banking and quality controls dedicated to all types of cells, generated from research laboratories, with a special focus on hPSC comprehensive chromosome screening. In line with recent literature, our results obtained from genomic characterization of hPSCs indicated that, besides long-term cultures, other procedures such as genome editing, cryopreservation and differentiation can contribute to the acquisition of genomic changes. Thus, to optimize time and resources, cells should periodically be monitored to check the presence of genetic variants and, at critical time points, specific in-process controls should be put in place since the earliest phases of project design. A great variety of technologies have been developed and are currently available to support researchers in genomic characterization of hPSCs. As a service facility, we performed genomic quality control of numerous hPSC clones using different methodologies, each of them characterized by individual technical advantages, drawbacks and requirements of specific competences. The results of our analyses suggest that combining conventional and array-based karyotype (i.e., array-based karyotype and array-based Comparative Genomic Hybridization) at specific time points provides effective and accurate hPSC genomic screening. In addition, PCR-based tests can also be instrumental to rapidly and cost-effectively monitor specific targets as recurrent aberrations.

Keywords: Human pluripotent stem cells, Genomic aberrations, Quality control

Poster: 963

MODELING DOMINANT OPTIC ATROPHY WITH INDUCED PLURIPOTENT STEM CELLS

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Dominant optic atrophy (DOA) is a rare progressive condition and the most common form of inherited optic neuropathy. This disorder is mainly caused by mutations in the nuclear gene OPA1, which encodes a mitochondrial protein with a key role in mitochondrial organization and dynamics. Mutations in OPA1 lead to the disarrangement of mitochondrial structure and a marked reduction in the ability to produce energy, causing degeneration of retinal ganglion cells (RGCs). This implies optic nerve atrophy and progressive loss of visual acuity, coming with blindness in many cases. Although most DOA cases are presented as a non-syndromic bilateral optic neuropathy, 20% of patients suffer from a syndromic disorder, with secondary symptoms affecting neuronal and muscular functions. This syndromic form with extra-ocular features is known as DOA 'plus'. Currently, there is no effective treatment for DOA due in part to the lack of an appropriated disease model. In this sense, the main objective of this study has been the implementation of induced pluripotent stem cells (iPSCs) technology to generate patient-specific iPSC-derived RGCs. These RGCs would allow to faithfully model these disorders in the affected cell target type of DOA, opening up the likelihood of designing a therapeutic approach. Up to now, an iPSC line was successfully generated in our laboratory from fibroblasts obtained from a DOA 'plus' patient harbouring the mutation c.1861C>T; p.Q621* in OPA1. The subsequent step has been the optimization of a protocol to obtain RGCs by differentiation of the patient iPSCs. This protocol is based on the modulation of several signalling pathways by mimicking embryonic development. Using this

protocol a homogeneous population of retinal progenitor cells has been obtained, followed by their final differentiation into RGCs, both in a control iPSC line and the mutant one. These RGCs could represent a valuable platform to delve into the pathophysiological mechanisms underlying DOA and for drug screening approaches.

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Keywords: Dominant optic atrophy, Disease model, Retinal ganglion cells

Poster: 964

ENRICHMENT OF HUMAN RETINAL GANGLION CELLS FROM IPSC RETINAL ORGANOIDS AS MODELS FOR STUDYING GLAUCOMA

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Neurodegenerative eye conditions are poorly understood, and treatment options are often insufficient to prevent blindness. The most common irreversible neurogenerative eye disease is glaucoma. The main characteristic feature of glaucoma is progressive retinal ganglion cells (RGC) loss. Even though increased intraocular pressure (IOP) has been established as a main contributor many patients with glaucoma progress to blindness without suffering from increased IOP. Those patients are referred to as normal tension glaucoma patients (NTG). In order to investigate cellular pathways disrupted in NTG patients contributing the RGC loss, induced pluripotent stem cells (iPSC) from NTGs and OHTs will be generated and subsequently differentiated into retinal organoids. We have recently optimized the procedures to extract RGCs via magnetic-activated cell sorting from retinal organoids derived from control iPSC. The purity of expanded RGCs in 2D cultures was analysed using cell specific markers such as CD90, Brn3a and RBPMS via qPCR and immunocytochemistry. Furthermore, electron microscopy was implemented to investigate the conditions of intracellular organelles. Future experiments will include functional assessment of the respiration capacity, metabolomics, transcriptomics and proteomics of RGCs extracted from retinal organoids derived from iPSC of NTGs and OHTs to precisely identify the differences and disease contributing factors in both patient groups compared to control individuals. This will lead to insights regarding the IOP independent disease mechanisms in glaucoma.

Funding Source: Velux Foundation BrainStem

Keywords: Glaucoma, Induced pluripotent stem cells, Organoids

Poster: 965

HUMAN URINE DERIVED STEM CELL: A POTENTIAL WINDOW INTO KIDNEY IMMUNE PATHOLOGIES

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Immune-mediated kidney pathologies remain one of the most common causes of renal dysfunction. However, the study of their pathogeneses remains confined to the invasive sampling of kidney biopsy and the use of animal models. The discovery of the renal origin of the urine mesenchymal stem cells (USCs) has opened a new avenue to study these cells' role within the kidney environment, particularly their immunomodulatory role. Here we report the effect of USCs and USC-derived extracellular vesicles (EVs) on T and B lymphocytes functions including activation, proliferation, cytokines and antibody secretion. In line with the previous reports using other mesenchyme stem cells, USCs negatively affect T cell functions. However, unexpectedly, we found that USCs have potent B cell stimulatory properties, mediated by the release of B cell survival signals like BAFF, CD40L and IL6, and inducing activation, proliferation, and antibody production by B lymphocytes. Characterization of USCs EVs showed they serve as carriers of these signals, having a similar effect on T and B cells. In addition, we found that USCs expressed receptors for the B cell stimulating agent, BAFF, suggesting a potential auto/paracrine role of this factor on the USCs. These cytokines are generally present within the germinal center of lymph node playing an essential role during the physiological, and pathological immune response. Our study findings suggest the possible role of USCs in kidney immune pathology, particularly in the formation of kidney tertiary lymphoid organs common in autoimmunity and organ rejection. Therefore, we propose that the phenotype, genotype and functional study of USCs could have the potential to be used as a non-invasive surrogate system for the diagnosis and risk assessment of kidney immunopathology.

Keywords: Kidney Immune pathology, Urine Stem Cells, B cell

Poster: 966

USING EQUINE EMBRYONIC STEM CELLS AS A MODEL TO INVESTIGATE INFLAMMATION IN TENDINOPATHY

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Tendon injuries occur commonly in human and equine athletes, and poor tendon regeneration leads to functionally deficient scar tissue which is prone to re-injury. Despite the prolonged recuperation periods and substantial welfare burden associated with tendinopathy, the mechanisms behind this dysregulated healing remain poorly understood. Previously, IL-1β was demonstrated to have adverse effects on the functional ability of adult tenocytes, yet these consequences could be rescued by using exogenous IL-1 receptor antagonist protein (IL1Ra). Nevertheless, in vivo, other inflammatory cytokines are present, and we evidence that IFN-γ, TNFα and IL-1β in combination have exacerbated deleterious effects on adult tenocytes that cannot be rescued by IL1Ra alone. These effects include altering tendon-associated gene expression and impairing the cells ability to contract a 3-dimentional collagen gel; a culture technique which much more closely resembles the in vivo environment. These in vitro methods have further been refined to determine whether bone marrow-derived mesenchymal stromal cells (BMSCs) release soluble factors which protect adult tenocytes from inflammation. In contrast to adult tenocytes, equine embryonic stem cell (ESC) derived tenocytes stimulated with IFN-γ, TNFα and IL-1β exhibit no detrimental effects on tendon-associated gene expression and generate tendon-like constructs in vitro indistinguishable from controls. To determine the mechanisms behind ESC-derived tenocytes inflammatory protection, we identified the inflammatory signalling pathways activated during tendinopathy. Using immunocytochemistry, we demonstrate that the proinflammatory transcription factor NF-κB is activated in adult tenocytes within one hour of stimulation with IFN-γ, TNFα and IL-1β. In contrast, inflammatory stimulation failed to activate NF-κB in equine ESC-derived tenocytes. Determining the key signalling cascades orchestrating tendinopathy using an in vitro ESC model will allow the development of targeted pharmaceutical interventions which minimise scar-tissue formation, resulting in diminished re-injury rates and improved quality of life.

Funding Source: This project is kindly funded by the Horserace Betting Levy Board.

Keywords: Inflammation, Tendinopathy, Regeneration

Poster: 967

USING EQUINE INDUCED PLURIPOTENT STEM CELLS TO UNDERSTAND THE GENETIC MECHANISMS BEHIND FRACTURE RISK

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Musculoskeletal disorders are associated with morbidity, health-care costs and an increased risk of mortality in humans. Horse and human chromosomes have strong shared synteny and the detailed breeding records and large family sizes of Thoroughbred horses provide an advantage to understand complex diseases. Bone fractures with non-traumatic origin are the main reason for euthanasia of Thoroughbred horses on the racecourse. Identification of the genetic factors and an understanding of the molecular pathways that they affect would allow the future development of new interventions to prevent, and possibly treat, fractures. A previous genome-wide association study (GWAS) showed significant genetic variation for fracture risk on chromosomes 9, 18, 22 and 31. The associated region on horse chromosome 18 contains 11 known genes that are involved in bone formation or fracture. This region corresponds to a highly conserved region on human chromosome 2. We have established an equine induced pluripotent stem cells (iPSCs) disease model to determine the biological pathways which are affected in high fracture risk. Skin fibroblasts from over 50 horses were genotyped, and classified as high and low genetic risk of fracture using a genome wide algorithm. The samples from the three highest and lowest risk horses were used to generate iPSCs and then differentiated into osteoblasts. We demonstrated that there are significant differences in the expression of Collagen type III (COL3A1) and signal transducer and activator of transcription 1 (STAT1). Furthermore, whole genome sequencing (WGS) data from fracture cases and controls revealed a possible causal SNP upstream of COL3A1. This SNP is associated with fracture risk and osteoblasts derived from horses that are homozygous for the SNP have a lower expression of COL3A1 than osteoblasts derived from wild-type or heterozygous horses. Luciferase assays demonstrated that the region containing the SNP has promoter activity and the SNP was predicted to affect SOX11 binding. SOX11 overexpression leads to a significant increase in COL3A1 demonstrating that COL3A1 may be implicated in fracture risk. Further step consists of genome editing in iPSC-osteoblasts by clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) in order to evaluate the effects of this SNP.

Funding Source: This work is kindly funded by the Horserace Betting Levy Board (HBLB)

Keywords: Induced Pluripotent Stem Cells (iPSCs), Single-nucleotide polymorphism (SNP), Bone fracture

Poster: 968

MODELING MCARDLE DISEASE USING IPSCS

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McArdle disease (glycogenosis type V) constitutes a rare autosomal recessive myopathy triggered by mutations in PYGM, the gene encoding the muscle isoform of glycogen phosphorylase (myophosphorylase). Around 150 pathogenic mutations have been described until date, and the transition c.148C>T; p.R50* represents the most frequent one in the Caucasian population. McArdle patients are unable to obtain energy from the muscle glycogen stores, eliciting an exercise intolerance usually accompanied by rhabdomyolysis and/or myoglobinuria. Currently there exists no treatment (although low to moderate exercise is strongly recommended), so the search for a therapy has been recognized as a priority. Induced pluripotent stem cells (iPSCs) are an excellent tool for the

generation of disease models, allowing a better understanding of the underlying molecular mechanisms and opening up the possibility to perform cell therapy applications and high-throughput drug screenings. The purpose of this study focuses on the establishment of a McArdle disease iPSCs model that can be used to develop a possible therapy in the near future. We have already generated two bona fide patient-derived iPSC lines harboring the homozygous mutation c.148C>T; p.R50*. In the next step, the iPSCs must be differentiated into the disease target cell type. The McArdle iPSC lines have been co-differentiated towards both skeletal muscle cells and motor neurons, as this strategy ensures an increased maturity of the myotubes. The selected differentiation protocol mimics the embryonic development with the addition of small molecules in the cell culture, activating or inhibiting key pathways to direct the cells to the desired lineages. The differentiated cultures do express mature myogenic markers like titin and desmin, and their functionality has been verified with the identification of calcium transients. Additionally, the disease model has been validated by proving the lack of myophosphorylase expression in the McArdle lines compared to controls, assessing as well the differences in terms of glycogen content.

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Keywords: McArdle disease, iPSCs model, Skeletal muscle

Poster: 969

EARLY IMPACT OF DYSTROPHIN DEFICIENCY DURING HUMAN SKELETAL MUSCLE GENERATION FROM PLURIPOTENT STEM CELLS

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Differentiation of induced pluripotent stem cells (iPSCs) into the skeletal muscle lineage has become a trusted model to study neuromuscular disorders (NMDs) in a human in vitro system. As they can recapitulate successive steps of muscle development in a dish, iPSCs give the opportunity to access the pathological events happening in embryos affected by NMDs, and potentially pinpoint early therapeutic targets immediately downstream of the mutation. Duchenne muscular dystrophy (DMD) phenotypes first manifest in children of about 3 to 5 years of age, but the initial disease mechanisms at the molecular level during muscle development are unknown, halting the development of early postnatal detection and therapies. In this project, we compared the myogenic differentiation dynamics of DMD and healthy iPSCs using single-cell transcriptomics and pseudotime analysis. We confirmed the expression of well-known regulators of myogenesis in defined clusters of cells expressing brachyury, Tbx6, Meox1, Pax3, Pax7, or Myog. Reconstruction of the developmental trajectory revealed that early after mesoderm induction and formation of somite progenitors, a majority of DMD cells shift to an alternative path on which myogenic regulators are strongly downregulated. Dysregulated genes include multiple factors involved in the formation of cellular junctions, such as cadherins, claudins and integrins. These families were also found dysregulated at an early somite stage in our comprehensive database containing RNA-seq data collected during myogenic differentiation of three additional DMD iPSC lines. At this time point, we detected two cell subpopulations characterized by their epithelial vs. mesenchymal morphology which were differentially represented in DMD cells, together with a distinct cell junction pattern. Finally, mutant cells showed an aberrant migration behavior when submitted to a scratch wound assay, suggesting possible functional consequences during the delamination of DMD somites. Altogether, these results highlight the value of single-cell transcriptomics and iPSCs for the early detection of genetic disease phenotypes.

Keywords: single-cell transcriptomics, Duchenne muscular dystrophy, skeletal muscle

Poster: 970

TISSUE ENGINEERING USING A NOVEL REPLICA MOLDING AND 3D PRINTING-BASED PLATFORM: IN VITRO GENERATION OF CONTRACTILE SKELETAL MUSCLES FROM TRANSGENE-FREE HIPSC-DERIVED MYOGENIC PROGENITORS

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For neuromuscular disorders, 90% of novel therapeutic strategies identified with traditional in vitro models fail in phase I clinical trials. To improve modelling, 3D tissue engineered skeletal muscles (TESMs) have been generated from primary myoblasts and from myogenic progenitor cells (MPCs) derived from human induced pluripotent stem cells (hiPSCs) with ectopic expression of Pax7. Primary cultures are known for their heterogeneity and limited self-renewal. Forcing hiPSCs into MPCs via transgene expression disturbs normal physiological processes resulting in a 4-fold lower contractile force when compared to primary myoblasts. To further improve modelling expandable transgene-free MPCs are preferred. We recently showed that purified transgene-free MPCs derived from hiPSCs can be expanded up to 5 x 10^11 fold while retaining differentiation capacity in 2D (van der Wal et al. 2018). We also developed a pipeline for fabrication of skeletal muscle tissue engineering devices using a simple 3D printing platform that can generate hundreds of devices a day without use of specialized equipment (Iuliano et al. 2020). Here we combined both platforms to assess the capacity of the hiPSC-derived MPCs to form TESMs. By optimizing culture conditions we found that our platform with 12 and 48 well configurations can support the formation of highly functional TESMs. TESMs

generated from 3 independent donors showed a specific force of 10-34 mN/mm2 which is comparable or higher than the specific force of primary myoblasts (12 mN/mm2). RNA and whole proteomic analysis showed a similar myosin heavy chain (MYH) expression profile with high expression of the embryonic MYH3 isoform in TESMs from both MPCs and primary myoblasts. We also tested whether our platform is suitable for drug testing by treating TESMs with small molecules known to affect skeletal muscle. We found that chloroquine induced a 15-fold reduction in contractile force while verapamil and cardiotoxin completely abolished contraction after 6 hours of incubation. A low dose of caffeine induced a 2-fold increase in contractile force. Based on contractile force, proteomics, and response to drug treatment we conclude that we have developed a platform using versatile 3D printing that supports the formation of high quality TESMs from transgene-free hiPSC-derived MPCs.

Keywords: Tissue engineered skeletal muscles, hiPSC-derived myogenic progenitor cells, Neuromuscular disorders

Poster: 971

SCALEABLE HUMAN SKELETAL MYOCYTES BY OPTI-OX REPROGRAMMING OF IPSCS FOR THE STUDY OF MUSCLE BIOLOGY, NEUROMUSCULAR JUNCTION AND METABOLIC DISORDERS

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Skeletal myocytes play roles in a number of biological processes ranging from limb movement to the regulation of nutritional homeostasis and are implicated in the pathophysiology of a variety of diseases 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, improving cellular reprogramming approaches for the differentiation of hiPSCs. Through targeting of genomic safe harbour loci, we used opti-ox to achieve homogenous, inducible expression of the myogenic regulator MYOD1. MYOD1 induction leads to shutdown of the core pluripotency network and activation of key myogenic factors including myosin heavy chain. Cryopreserved ioSkeletal Myocytes homogenously 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. ioSkeletal Myocytes produce a highly pure Myosin Heavy Chain positive population of cells within 4 days of thawing and are amenable to high-throughput screening pipelines. Demonstrating the value of the ioSkeletal Myocytes for muscle functional studies, Airyscan z-series stacks reveal structural proteins in a striate structure that contract in response to electrical stimulation and increased extracellular potassium levels. Critically for metabolic studies, robust expression of the insulin-regulated glucose transporter GLUT4 is also detected. The scalability and robustness of opti-ox reprogramming and ease of use of the ready to culture ioSkeletal Myocytes provide a unique hiPSC based model for research of muscle biology, including disease modelling and HTS applications.

Keywords: Musculoskeletal, Reprogramming, Muscle

Poster: 972

HUMAN IPSC DERIVED CELL CULTURES TO STUDY NEURONAL DYSFUNCTIONING AND MYELINATION IN BRAIN WHITE MATTER DISORDER 4H LEUKODYSTROPHY

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4H leukodystrophy is an inherited brain white matter disease with a progressive disease course and no treatment option. Ataxia and delayed motor development are common presenting symptoms, and MR imaging shows hypomyelination and cerebellar atrophy in the majority of patients. 4H is caused by mutations that affect RNA polymerase III (POLR3), but disease mechanisms are poorly understood. Recent studies have identified atypical 4H patients without hypomyelination, but with basal ganglia abnormalities, pointing to a central role for neurons in 4H pathomechanisms. To study disease mechanisms in 4H, we generated induced pluripotent stem cells (iPSCs) from 4H patients and controls and differentiated the iPSCs into cerebellar neurons. Although the generation of cerebellar cells was normal in 4H, RNA sequencing revealed a number of changes in gene expression. One of the top candidates was ARX, which showed a significantly decreased expression in 4H cells. ARX is known to be important for the development of cortical interneurons. To study 4H neurons in more depth we differentiated iPSCs into cortical neurons with glutamatergic and GABAergic identity. Preliminary data confirmed a decreased expression of ARX in 4H cortical neurons compared to controls that also correlated to a decrease in GABAergic synapses. Currently we study whether affected interneuron development plays a role in hypomyelination in 4H. We make use of an in-house developed myelinating co-culture of human iPSC-derived neurons and human iPSC-derived oligodendrocytes. This co-culture allows automated microscopy and the study of cell-autonomous effects. To summarize, we have identified abnormal expression of ARX in 4H cerebellar cells and cortical neurons. In line with ARX function in cortical interneuron development, we show that the number of GABAergic synapses was decreased in 4H cultures. Next, we will test whether myelination of these cortical neurons is affected in 4H cultures. The results of this

study increase our understanding of the affected cell types and disease mechanisms in 4H leukodystrophy, which is essential for future therapy development.

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Keywords: Neuronal development, Neuron-glia interactions, Myelin

Poster: 973

SOX9-INDUCED GENERATION OF FUNCTIONAL ASTROCYTES SUPPORTING NEURONAL MATURATION IN AN ALL-HUMAN SYSTEM

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Astrocytes, the main supportive cell type of the brain, show functional impairments upon ageing and in a broad spectrum of neurological disorders. Limited access to human astroglia for pre-clinical studies has been a major bottleneck delaying our understanding of their role in brain health and disease. We demonstrate here that functionally mature human astrocytes can be generated by SOX9 overexpression for 6 days in PSC-derived neural progenitor cells. Inducible (i)SOX9-astrocytes display functional properties comparable to primary human astrocytes. Importantly, electrophysiological properties of iNGN2-neurons co-cultured with iSOX9-astrocytes are indistinguishable from gold-standard murine primary cultures. The high yield, fast timing and the possibility to cryopreserve iSOX9-astrocytes without losing functional properties makes them suitable for scaled-up production for high-throughput analyses. Our findings represent a major step forward to an all-human iPSC-neural model for drug development in neuroscience and towards the replacement of animal use in biomedical research.

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Keywords: Induced pluripotent stem cells, Astrocytes, all-human co-culture system

Poster: 974

CO-CULTURE OF HIPSC-DERIVED NEURONS AND CARDIOMYOCYTES IN COMPARTMENTALIZED MICROFLUIDIC DEVICE

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In the human body, heart is mostly innervated by autonomic nervous system. The innervation of heart begins in the early stages of embryogenesis and can be considered as a co-maturation. In other words, the specification and growth of innervating neurons are regulated by cardiomyocytes whereas maturation of cardiomyocytes is enhanced by the neuronal signals. The interferences in heart innervation can lead to cardiac diseases making the study of heart innervation and interactions between neurons and cardiomyocytes an important topic. In addition, the co-cultures of neurons and cardiomyocytes produce valuable information of the role of innervation to the heart development. In this study, human induced pluripotent stem cell (hiPSC)-derived neurons and cardiomyocytes were co-cultured in compartmentalized microfluidic device originally designed for axonal isolation studies. This device isolated neuronal somas in their own compartment, allowing only the axons to grow through microtunnels to the cardiac compartment. In addition, the cell-specific microenvironment was created by using cell-specific medium. Immunocytochemical staining, RT-qPCR and video microscopy analysis of beating cardiomyocytes were used to analyze interactions between neurons and cardiomyocytes. Moreover, the functional interaction of the two cell types was determined by exposing neurons to high K+ solution. According to the results, both cell types were viable in the co-culture and the axons grew alongside the cardiomyocytes in their compartment. Preliminary evidence of interactions between neurons and cardiomyocytes was seen with the immunostainings and video microscopy analysis. Additionally, the study showed that the designed microfluidic device has potential for various in vitro modeling applications.

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Keywords: Neurons, Cardiomyocytes, Body-on-Chip

Poster: 975

EXOSOMES PROMOTE THE RECOVERY FROM SYNAPTIC DYSFUNCTION IN HUMAN HUNTINGTON S DISEASE IPSC-DERIVED STRIATAL-LIKE NEURONS

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Multiple mechanisms have been implicated in the pathogenesis of Huntington's disease (HD), a disorder caused by an expansion of CAG repeats in the HTT gene. Recent studies reveal that synapse dysfunction precedes cell death by many years, primarily affecting the medium-sized spiny neurons in the striatum. Also, emerging evidence support that GABAergic dysfunction contributes to HD pathogenesis. iPSC technology and striatal-like neurons used for disease modelling has given new insights into disease mechanisms. In this study we explored the effect of exosomes isolated from human fibroblasts on the electrophysiological and morphological alterations observed in mature medium spiny-like neurons differentiated from HD and control iPSC. Exosomes were isolated from cell culture media of human fibroblasts using ultracentrifugation method and were added to cell culture media for 5 days. iPSC-derived striatal-like neurons expressed selective identity, at day 45, confirmed by the expression of striatal GABAergic markers, namely GAD65/67 and DARPP-32. At day 60, mature neuronal cells were confirmed through increased MAP2+/BIII-tubulin+ ratio; DARPP-32+ neurons were observed as neurons became more mature, mostly in control cells. At day 80, full maturation stage was reached, as most cells were positive for SMI-32 neuronal marker and both HD and control derived neurons displayed action potentials and Na+- and K+-channels. Whole-cell patch-clamp recordings showed that HD neurons display lower frequency and amplitude of mIPSC and lower GABA receptor-mediated current density. Exposure of HD neurons to exosomes from human control fibroblasts induced a normalisation to control levels in the total GABAergic currents. In addition, a normalisation in the frequency, but not in amplitude, of mIPSC was also observed, indicating that these alterations are mediated by a rise in synaptic contacts. In conclusion, we clearly show that HD iPSC-derived neurons are capable of forming functional synapses and participate in circuits but display GABAergic abnormalities that can be normalized by exosome treatment. These findings suggest that delivery of exosomes derived from control human fibroblasts might be a potential therapeutic strategy for the future treatment of HD.

Funding Source: COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT, under project POCI-01-0145-FEDER-029621 and UIDB/04539/2020.

Keywords: HD iPSC-derived striatal-like neurons, GABAergic currents, exosomes

Poster: 976

BUILDING HUMAN NEURO-VASCULAR INTERACTIONS IN MICROPHYSIOLOGICAL ENVIRONMENT

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The nervous system relies on electrochemical signals to transfer information and control the functions of human body, while vascular networks provide oxygen and nutrients for the cells in all tissues. Both neuronal and vascular networks are crucial for maintaining the homeostasis in humans. These two network systems cover the whole human body and are abundantly present in various types of tissues. A stable 3D neuro-vascular co-culture is needed for establishing more in vivo–like innervated and vascularized tissue models in eg. Body-on-Chip (BOC) research. Here, we used human induced pluripotent stem cell (hiPSC) –derived neural progenitor cells, human umbilical vein endothelial cells (HUVECs) and either human adipose stem/stromal cells (hASCs) or bone marrow–derived stem/stromal cells (BMSCs) as a mural cell type to create 3D neuro-vascular co-culture model. Various hydrogels or their combinations were used in AIM Biotech 3D Cell Culture Chip to establish 3D cell culture. The chip has three replicate hydrogel regions which are all flanked by two media channels. Interstitial flow was created to the chip by adding different volumes of cell culture media to the media reservoirs. Different sequential cell plating set-ups were tested to find an optimal co-culture model. The cultures were then maintained for up to 14 days. Co-cultures were analyzed with real time imaging and immunocytochemical staining. In the study, we showed that the studied cell types connected with each other and formed partly aligned structures. We also noticed a need for supporting mural cell type, which is especially crucial in establishing mature vascularization. By using a microfluidic chip, we observed that neurons tolerate interstitial flow and grow neurites inside the hydrogel channel. Furthermore, finding an optimal microenvironment for all cell types is critical, as we detected rapid degradation of particular hydrogels caused by neuronal cell activity. Here, we present a novel human neuro-vascular model that is applicable in creating in vivo-like tissue models and in revealing mechanisms in neuronal-vascular interactions.

Funding Source: Centre of Excellence in Body-on-Chip Research is funded by Academy of Finland.

Keywords: Neurogenesis, Angiogenesis, Microphysiological systems

Poster: 977

DIRECT CONVERSION OF HUMAN FIBROBLASTS TO ASTROCYTES

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Astrocytes are essential cells in normal brain function and are emerging as key players in neurological disease. Traditionally, studies have been performed using rodent astrocytes but these are considerably less complex compared to their human counterpart. Over the recent years we and others have shown that it is possible to generate astrocytes from human pluripotent stem cells for disease modeling. However, during reprogramming to pluripotency the epigenetic landscape of donor cells is reset leaving the iPSCs at an embryonic-like state. Thus, when modeling sporadic and late-onset diseases potential important information might not be revealed due to the cells inability to properly mimic the age of a patient or epigenetic components to disease. Studies of neurons obtained through direct conversion of fibroblasts, in which the donor cells bypass a pluripotency stage, have shown that the cellular age is preserved. However, only few studies have explored the generation of human astrocytes through direct conversion and efficiency as well as characterization of the obtained cells remains insufficient. Here we describe efficient direct conversion of human embryonic

fibroblasts to functional induced astrocytes (HEF-iAs). Through lentiviral-mediated overexpression of the transcription factors Sox9, Nfia and Nfib we obtain HEF-iAs that have a morphology that resemble primary human astrocytes, express astrocytic proteins and have a gene expression profile similar to that of primary human fetal astrocytes. Furthermore, HEF-iAs has the capacity to take up glutamate and form functional gap junctions, key functions of astrocytes. Interestingly, optimisation of the conversion conditions significantly increased yield of HEF-iAs. Importantly, this also improved the conversion of human adult fibroblasts to astrocytes, a necessity to enable disease modeling of age-related neurological diseases. In addition, we can for the first time show electrophysiological analysis of a co-culture system of neurons and astrocytes obtained through direct conversion from the same starting fibroblast culture. Direct conversion of human fibroblasts to astrocytes have the potential to add a higher dimension of complexity to current in vitro human astrocyte models and become useful for modeling late-onset neurological disorders.

Funding Source: Stiftelsen Sävstaholm, Vetenskapsrådet, Hjärnfonden, Craafoorska stiftelsen and Hardebo stiftelse

Keywords: Direct conversion, Astrocytes, Reprogramming

Poster: 978

KNOCKDOWN OF SPECIFIC KINASES LEADS TO A MORE EFFICIENT DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO NEURAL CREST CELLS

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Human induced pluripotent stem cells (hiPSCs) can be differentiated into various cell types. One of them are neural crest cells (NCCs), which are multipotent precursors of cells, that contribute to skin pigmentation, development of the cornea, the peripheral nervous system and the craniofacial skeleton. Due to their differentiation potential, NCCs are an ideal model system for developmental studies. There are several protocols to differentiate hiPSCs into NCCs. Here, we have established an effective differentiation protocol by adapting and extending the protocol from Callahan et. al. (2016), combined with siRNA transfection. By screening several kinases, we found four kinases that exert a significant effect on the differentiation of hiPSCs to NCCs. The knockdown of two of these kinases for 48h at the beginning of the differentiation process resulted in an enhanced differentiation of hiPSCs to NCCs. This new protocol enables the establishment of a simple and fast system to study developmental aspects and might be useful in regenerative medicine.

Keywords: human induced pluripotent stem cells, neural crest cells, differentiation

Poster: 979

IPSC-DERIVED MICROGLIA FOR DISEASE MODELING OF FRONTOTEMPORAL DEMENTIA 3

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Frontotemporal dementia (FTD) is a common cause of early-onset dementia, with no currently available cure. Research is therefore crucial to elucidate disease mechanisms, to facilitate new therapeutic interventions. FTD3 is a rare form of the disease, caused by a point mutation in the charged multivesicular body protein 2B (CHMP2B). This mutation has been observed to cause neuronal phenotypes, such as mitochondrial defects and abnormal endosomal-lysosomal fusion. However, the role of glial cells in FTD3 pathogenesis has until recently been rather unexplored. Neuroinflammation has been identified in most neurodegenerative disorders, with microglia potentially acting as proinflammatory drivers of the diseases. To assess the role of microglia in FTD3, we have derived induced pluripotent stem cells (iPSC) from a healthy individual, and by using CRISPR/Cas9 gene editing, established new knock-in cell lines, either heterozygous or homozygous for the CHMP2B mutation. These iPSC have been differentiated into both microglia and neurons, to observe how the mutation affect the two cell populations, and the microglia-neuron interaction. We have evaluated the proinflammatory profile of these microglia, performed metabolic tests, and investigated the microglial effect on neurons, to identify differences between our mutated cell lines and healthy control, and thus identify new disease phenotypes, specific for microglia. These findings have been further verified by RNA sequencing.

Funding Source: Novo Nordisk Foundation (GliAD – NNF1818OC0052369), Innovation Fund Denmark (BrainStem and NeuroStem), Alzheimerforeningen

Keywords: FTD3, iPSC, Microglia

Poster: 980

IMPROVED METHODOLOGY FOR THE GENERATION OF FUNCTIONAL OLIGODENDROCYTES FROM HPSCS

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Oligodendrocytes (OLs) are highly specialized cells of the central nervous system (CNS) responsible for myelin production and metabolic support of neurons. Defects in OLs are crucial in several neurodegenerative diseases including multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Scarce access to

primary samples and lack of efficient protocols to generate OLs from human pluripotent stem cells (hPSCs) are hampering our understanding of OL biology and the development of novel therapies. To promote the conversion of hPSCs into OLs, we have screened for a number of transcription factors (TFs) previously reported to be involved in OL generation. For this, hPSCs were fated for 8 days toward neural progenitors, and then transduced with an inducible lentiviral vector encoding for the different TFs. We found that the overexpression of SOX10 was sufficient to generate O4+ oligodendrocyte precursor cells (OPCs) from hPSCs only 10 days after SOX10 induction. Generated OPCs expressed mature OL proteins as MBP or MOG. At the transcriptome level, generated OPCs resembled primary OPCs. To date, OPCs have been derived from eight different hPSC lines including those derived from patients with MS and ALS. To test the functionality of generated OPCs, O4+ cells were co-cultured together with hPSC-derived neurons for additional 20 days, finding that O4+ cells were able to myelinate the neurons. Moreover, O4+ cells were injected intracerebrally in newborn shiverer RAG2-/- mice and the tissue was examined 16 weeks later, finding that generated OLs extended within the corpus callosum and generated functional myelin, demonstrating the functionality of generated cells also in vivo. The protocol also describes an alternative for viral transduction, by incorporating an inducible SOX10 in the safe harbor locus AAVS1, yielding ~100% pure OPCs. O4+ OPCs can be co-cultured with maturing hPSC-derived neurons in 96/384-well- format plates, allowing the screening of pro-myelinating compounds. We have developed a novel methodology for a fast (20 days from hPSC stage) and efficient generation of functional OLs, which allow testing of compounds involved in myelination. This technology will allow further studies to better understand human OL biology and the screening of potential compounds involved in myelination in a human setting.

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Keywords: neural differentation, oligodendrocytes, glial cells

Poster: 981

AXONS ON CHIP MODEL FOR STUDYING THE SPREAD OF AGGREGATED ALPHA SYNUCLEIN AND ITS EFFECTS ON NEURONAL FUNCTIONALITY

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the accumulation of aggregated protein alpha synuclein (AS) in neurons. AS aggregates are shown to have prion-like spread in neuronal networks, which is correlated with the impairment of network activity and mitochondrial dysfunction. Human pluripotent stem cell (hPSC) -derived neuronal cells cultured on compartmentalized microfluidic devices can provide a highly applicable tool for studying the spread of AS pathology in vitro. In this study, we used a polydimethylsiloxane (PDMS) microfluidic device, which contains three sequential compartments interconnected by microtunnels. Separated compartments provide axonal isolation in a controllable microenvironment, making it possible to study the essential cellular mechanisms behind the AS aggregate propagation. The large middle axonal compartment enables focusing on the isolated axons for thorough analysis. The axons, however, tend to grow in random directions in such a large compartment without axonal guidance; thus, lacking interconnectivity between compartments. We recently showed that such connectivity can be achieved by integrating the device with a topographically patterned nanostructural surface. Here, we optimized the seeding protocol for exogenous preformed AS fibrils (PFF) compatible with the axonal device. We observed evidence that PFFs can seed the AS aggregate accumulation in hPSC -derived neuronal cell networks and the spread of aggregates is detectable in the isolated axons. The utilized device with integrated topography can provide an effective in vitro model for studying the cellular mechanisms of AS aggregate propagation in a neuronal network.

Funding Source: This study was supported by the Academy of Finland, Centre of Excellence in Body-on-Chip research (#312414 and #312411), Academy of Finland (#332693) and Orion Research Foundation (grant for postdoctoral research 2019).

Keywords: Parkinson's disease in vitro model, microfluidics, hPSC-derived neuronal networks

Poster: 982

DEVELOPMENT AND CHARACTERISATION OF PATIENT IPSC-DERIVED ECTODERMAL-MESODERMAL ASSEMBLOIDS TO INVESTIGATE RNA-TARGETING THERAPIES IN HUNTINGTON'S DISEASE

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Advances in organoid technology have led to the development of 'assembloids'; modular organoids consisting of cell types of different developmental origins. Combining a mesodermal organoid with an ectodermal cortical organoid results in the integration of mesodermal-lineage cells such as microglia and endothelial cells into the neuronal cell network. As such, assembloids have a high degree of complexity and can be used as a high-fidelity representative in vitro model of neurodegenerative disease. Huntington's disease (HD) is an inherited progressive neurodegenerative disorder caused by polyglutamine expansion mutations in the huntingtin protein that result in both toxic gain-of-function (through protein aggregation) and loss-of-function. There are currently no therapies for HD; however a potential therapeutic avenue is to use antisense oligonucleotides (AONs) to regulate splicing to control protein aggregation. Here, we used two HD patient-derived iPSC lines together with two age- and sex-matched control iPSC lines to create ectodermal-mesodermal assembloids to investigate the cellular neuropathology of HD. Assembloids were created by fusing neuronal embryoid bodies with mesodermal-patterned embryoid bodies before encapsulation in Matrigel and long-term culture. Assembloids were collected at regular intervals to track development. Preliminary data showed no gross morphological

or size differences between control and patient assembloids during development. However, immunofluorescent analysis showed decreased cortical plate formation and cytoarchitecture changes in patient assembloids. Assembloids showed integration of lineage distinct cell types (i.e. microglia into the neuronal module) and developed an endothelial cell network. We are currently investigating the potential of assembloids to model known HD phenotypes such as protein aggregation and mitochondrial dysfunction, as well as study neuronal activity via multielectrode array and calcium signalling. Furthermore, preliminary data with a fluorescently-labelled AON showed that AON is capable of trafficking to the nucleus of neurons of assembloids. Overall, our model will help reveal cellular phenotypes in HD and will serve as platform for testing potential HD therapies such as AONs.

Funding Source: This work was supported by funding from Campagneteam Huntington.

Keywords: Huntington's disease, organoids, antisense oligonucleotides

Poster: 983

HUMAN RAPHE-TYPE ORGANOID TO MODEL THE ROLE OF SEROTONIN ON CORTICAL DEVELOPMENT

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Serotonergic neurons are located in the raphe nuclei of the hindbrain from where they project throughout the brain and release the neurotransmitter serotonin. Despite the well-known role of serotonin as a neuro-behavior modulator, emerging evidence point to an early function in the developing brain including progenitor cell expansion, neuronal migration and axonal guidance. However, the molecular effects of serotonin during early human brain development are poorly understood. In this context, human induced pluripotent stem cells (iPSC)-derived cerebral organoids, which faithfully recapitulate certain aspect of early human brain development in vitro, have emerged as an attractive tool. In this project, we developed a protocol to generate raphe-type organoids and studied the role of serotonin on human forebrain development. More specifically, we established raphe-like organoids through small molecule-driven modulation of signaling pathways like Wnt and SHH, which are known to be involved in the development of central serotonergic neurons in vivo. At early time points, raphe-like organoids are composed of progenitor cells which exhibit characteristic regional markers including NKX2.2 and NKX6.1 and show a subsequent activation of FOXA2. Upon terminal differentiation, matured organoids display increased amounts of serotonergic neurons expressing serotonin and the synthesizing enzyme TPH2. When investigating the role of serotonin on forebrain progenitor cell behavior, we observed an increase in cell proliferation. By applying specific serotonergic receptor agonists and antagonists, we identified the 5-HT2C receptor as mediator of the enhanced proliferative activity. When fusing raphe-type with forebrain-type organoids, we developed a model to study the projections of serotonergic neurons towards the cortical areas. Our human iPSC-derived, three-dimensional fusion model enables us to further decipher the role of serotonin in cortical development on the molecular level and open the possibility to study associated diseases.

Keywords: Serotonin, Cerebral organoid, Raphe organoid

Poster: 984

THE PSYCHEDELIC PSILOCIN FOSTERS NEUROPLASTICITY IN IPSC-DERIVED HUMAN CORTICAL NEURONS

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The serotonergic plant-hallucinogen psilocybin is studied as innovative medication in anxiety, substance abuse and treatment-resistant depression. Animal studies show that psychedelics promote neuronal plasticity by strengthening synaptic responses and protein synthesis. However, the exact molecular and cellular changes induced in the patient's brain are not entirely understood. Here, we treated cortical neurons derived from human induced pluripotent stem cells (iPSC) with the psychoactive 5HT2A receptor agonist psilocin. We analyzed pre-and postsynaptic markers, pathways related to neuroplasticity and 5HT2A receptor localization. Acute exposure led to a decrease in axonal extracellular 5HT2A receptor presentation which may indicate receptor complex formation or internalization. We further found the number of presynaptic BDNF, SV2A, Synaptophysin and postsynaptic PSD-95 puncta to be increased twenty-four hours after ten minutes 10 µM psilocin exposure. Synaptophysin, BDNF and phosphorylated TrkB (activated BDNF receptor) protein level was elevated as well. The pro survival Akt pathway was activated, marked by an increase in phosphorylated protein state. Modulation of the axon initial segment and upregulation of activity-related immediate early genes, like cFOS and hallucinogen-specific EGR-2 were indicative for an altered excitability. That was accompanied by a strong increase in Ca2+ transients after acute (ten minutes) psilocin administration. Chelerythrine (PKC inhibition) and Dynasore (inhibition of clathrin-coated vesicle invagination) abrogate psilocin-induced BDNF increase suggesting a PKC - and endocytosis mediated process. Co-treatment with the selective 5HT2A receptor antagonist Ketanserin blocked the aforementioned effect, indicating a receptor involvement. These data suggest that exposure of human neurons to psilocin provokes a dynamic cascade which might induce a state of enhanced neuronal plasticity. This neuroplasticity booster could explain why psilocin is beneficial in the treatment of neuropsychiatric disorders where synaptic dysfunctions are discussed.

Keywords: Psychedelic, Cortical Neurons, Neuroplasticity

Poster: 985

IMPLICATIONS OF THE VAL66MET POLYMORPHISM OF THE BDNF GENE ON NEURONAL MORPHOLOGY AND FUNCTION USING HUMAN IPSC-DERIVED NEURONAL CULTURES

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Brain-derived neurotrophic factor (BDNF) is involved in a multitude of processes that are important for brain development including neuronal survival, neurite outgrowth or synaptic plasticity. An estimated 30 - 50 % of the population is homozygous or heterozygous for a single nucleotide polymorphism (rs6265) in the BDNF gene which causes a substitution of valine (Val) to methionine (Met) at codon 66 in the pro-domain of BDNF (Val66Met). Animal overexpression models indicate that the modification of the BDNF protein impairs the intracellular trafficking, as well as activity-dependent release of BDNF. The polymorphism was also associated to psychiatric disorders such as schizophrenia or major depression. We set out to investigate the effects of the Val66Met polymorphism on BDNF trafficking, neuronal morphology, and function in human induced pluripotent stem cell (iPSC)-derived neuronal cultures generated from healthy donors homozygous for either the Val or Met variant of the gene. To account for the given genetic heterogeneity of humans, we additionally generated isogenic cells lines. IPSC were differentiated into homogeneous cultures of cortical neurons expressing cortical layer-specific transcription factors. When comparing neurite outgrowth from neuronal cultures at early developmental stages we observed a significant reduction in neurite lengths accompanied by a significant reduction in the number of branching points in neurons derived from Met/Met carriers. Sholl analysis at later stages showed a reduction in number of intersections in neurons carrying the Met-BDNF variant. When investigating BDNF trafficking, we observed a decreased number of BDNF + vesicles on neurites of Met/Met carriers compared to Val/Val neurons. Additional experiments should decipher, in how far the polymorphism affects neuronal function and network activity. Together, our data give first experimental evidence that the exchange of the amino acid Val to Met in the human BDNF gene has measurable implications in human neuronal development.

Keywords: BDNF, Val66Met polymorphism, cortical neurons

Poster: 986

DECIPHERING THE ROLE OF OSTEOCRIN IN THE PATHOGENESIS OF SCHIZOPHRENIA

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About 21 million people worldwide are suffering from schizophrenia (SCZ). This neuropsychiatric disease is a multifactorial disorder with high heritability that is characterized by hallucinations, lack of motivation and memory deficits. On the molecular level, an imbalance of neurotransmitters and changes in the content of synaptic proteins and receptors have been suggested to critically contribute to the pathogenesis of SCZ. However, the exact pathomechanisms underlying SCZ remain elusive. While osteocrin (OSTN) is mostly described for its role in bone growth as well as muscle endurance, recent report showed that OSTN has been evolutionary repurposed to be expressed in the CNS of some primates including humans. Interestingly, OSTN expression is regulated by the transcription factor MEF2C in an activity-dependent manner and affects neuronal morphology, by reducing neuronal ramification. Whereas MEF2C has been associated with SCZ, a link between OSTN and schizophrenia has not been described. We identified a patient suffering from SCZ with a heterozygous deletion of chromosome 3q28 where the gene encoding for the peptide OSTN is localized. Neurons generated from induced pluripotent stem cells (iPSCs) from this patient are characterized by changes in neuronal morphology compared to the unaffected first-degree relative. Specifically, Sholl analysis of neurons differentiated for about 50 days in vitro show a significant higher level of neurite ramification compared to control neurons. In addition, we identified differences in the length and distance to the soma of the axon initial segment, indicating a different level of neuronal excitability. Our data link the expression of OSTN to neuronal morphology and function in schizophrenia patients' neurons and might qualify as a molecular target for therapy.

Keywords: schizophrenia, osteocrin, cortical neurons

Poster: 987

HUMAN IPS CELL-DERIVED MICROGLIA - TOWARDS MODELING SYNAPTIC PRUNING-ASSOCIATED CHANGES IN SCHIZOPHRENIA

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Schizophrenia is a heritable psychiatric disorder which affects approximately 1% of the world population. The disease is characterized by a range of symptoms such as hallucinations, lack of motivation and depression, as well as cognitive impairments. The most significantly associated genetic locus for schizophrenia identified in genome-wide association studies lies within the major histocompatibility complex region and involves structurally distinct alleles of the complement component 4 (C4) genes. Thereby, alleles leading to a higher expression of C4A were correlated to an increased risk for schizophrenia. The complement cascade has been shown to play an important role in microglia-mediated elimination of synapses during development. Given the reduced numbers of synapses in brains of individuals with schizophrenia, it has been suggested that excessive complement activity and synaptic pruning contributes to the development of the disease during late adolescence and early adulthood. In this project we set out to analyze synaptic pruning in an induced pluripotent stem cell (iPSC)-derived in vitro model. We established a differentiation protocol which allows the generation of human microglia-like cells in high purity. These cells express key microglia markers, they have a high phagocytic capacity and respond to pro-inflammatory stimuli. In order to investigate potential differences in synaptic pruning capacity and activity we generated a cumate-inducible

C4A gene expression system and established specific co-culture conditions with mature iPSC-derived cortical neurons. We expect that this experimental setting will allow to determine genotype-phenotype relationships with respect to synaptic pruning and C4A expression and will serve as a potential model for drug discovery.

Keywords: schizophrenia, microglia, synaptic pruning

Poster: 988

NEW LUMINESCENT CELL-BASED ASSAY TO DETECT BOTULISM NEUROTOXINS USING HUMAIN INDUCED PLURIPOTENT STEM CELL DERIVED MOTOR NEURONS

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Botulinum neurotoxins (BoNTs) are metalloproteases produced by Gram-positive bacterium Clostridium botulinum. Modern recombinant BoNTs (rBoNTs) can be produced using Escherichia coli as the host. BoNTs preferentially target peripheral motor neurons and block the release of acetylcholine by cleaving members of the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) complex, essential for neurotransmitter release. This leads to muscular relaxation and flaccid paralysis. These toxins may be used as pharmaceutical agents for the treatment of many neurological diseases, as well as for aesthetic applications. Each new batch of pharmaceutical BoNTs must be tested for BoNT potency. For years, the gold standard assay to test BoNT pharmaceuticals was the mouse lethality bioassay. To avoid the use of animals in these tests, several in vitro assays based on cells have been developed. Most of them include rodent cells and immortalized cell lines which have limitations concerning accuracy and physiological relevance. To improve these assays, we developed a new cell-based assay using human induced Pluripotent Stem Cells (hiPSC)-derived motor neurons. The assay described here detects the activity of BoNTs, targeting the synaptosomal-associated protein of 25 kDa (SNAP-25) and combines a toxin-sensitive hiPSC-derived motor neurons model with an engineered detection system using a split NANOLUC™luciferase. In this approach, a single chain polypeptide composed by a split luciferase which is held together with a linker containing SNAP-25, is incorporated into human motor neurons. Once BoNT enters into the cell and cleaves its target, the two components of the NANOLUC™luciferase are separated and thus cannot generate luminescent signal. In this system, BoNT potency is inversely correlated with the cleavage of the engineered luciferase. The results presented here show a convenient and rapid (9-day) cell-based assay. Moreover, our assay was able to discriminate the potency of different rBoNTs and was sufficiently sensitive to detect femtomolar concentrations of BoNT. In addition, we showed that our test can also be used to study the different steps of BoNT intoxication, such as toxin binding and translocation.

Keywords: Botulinum neurotoxin, cell-based assay, human induced pluripotent stem cell

Poster: 989

EXPLORING THE HIPSC-DERIVED NEUROSPHEROID MODEL TO STUDY ASTROCYTE ACTIVATION DURING NEUROINFLAMMATION

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Neuroinflammation is associated with pathogenic processes and disease states [1]. Upon inflammatory stimuli, microglia and astrocyte activation contribute to tissue healing and restoration of CNS homeostasis. Nonetheless, excessive glial activation can cause neuronal death and chronic neuroinflammation. Much remains unknown in what concerns: i) the molecular mechanisms that trigger and sustain astrocyte activation; ii) the effector molecules of the downstream microenvironment remodelling. Experimental models in which the human neural cells and their microenvironment are represented will be key to study such processes. This work aims to establish and explore a human cell model of neuroinflammation to study astrocyte activation. With this purpose, we explored hiPSC-derived neurospheroids, a methodology pioneered by our team. hiPSC-derived neural progenitor cells are differentiated in perfusion stirred-tank bioreactors into neurospheroids composed of neurons, astrocytes, and oligodendrocytes. We showed that neurospheroids recapitulate specific features of the brain microenvironment, such as brain-like ECM deposition and neuron-glia interactions. Herein, we challenged neurospheroids with pro-inflammatory factors reported to induce activation of astrocytes in mice models (TNF-α, IL-α, and C1q). Upregulation of neuroinflammatory genes (e.g., SERPINA3 and C3), concomitant with the secretion of pro-inflammatory cytokines, such as MCP-1 and IL-8, along the 72h of insult, suggested activation of astrocytes. Challenged astrocytes also displayed an impaired capacity to uptake glutamate and to secrete glutamine in comparison to the unstimulated control, suggesting functional impairment. We also observed increased neurite outgrowth of β-III tubulin-positive neurons on a laminin-rich substrate, suggesting ECM remodelling events. Transcriptomics and proteomics profiling of neurospheroids is ongoing. The results indicate that pro-inflammatory factors induce astrocyte activation in neurospheroids, recapitulating transcriptional changes and functional impairment, hallmarks of neuroinflammation. Therefore, the human neurospheroid model can be a useful tool to dissect neuroinflammatory mechanisms associated with diseases.

Funding Source: iNOVA4Health Research Unit (UIDB/04462/2020), funded by Fundação para a Ciência e Tecnologia (FCT)/ Ministério da Ciência e do Ensino Superior (MCTES) and by FEDER ; FCT/MCTES, project PTDC/BTM-ORG/29580/2017

Keywords: neuroinflammation, 3D cell models, microenvironment

Poster: 990

DEXAMETHASONE TRIGGERS IRON-DEPENDENT ALPHA-SYNUCLEIN EXPRESSION IN PARKINSONS DISEASE DERIVED BRAIN ORGANOIDS

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Excessive accumulation of alpha-synuclein (a-syn) predisposes to the development of Parkinson's disease (PD), a disorder characterized by neurodegeneration in the substantia nigra and concomitant motor impairments. It was previously shown that stress-induced release of glucocorticoids accelerates the progression of PD and that the glucocorticoid receptor (GR) is downregulated in several neurodegenerative as well as in stress-related diseases. The impact of altered a-syn protein levels on GR dysfunction and stress-related protein expression is largely unexplored, but may have severe implications for PD manifestation and disease progression. Therefore, we examined the effect of chronic stress in two models overexpressing human a-syn: a transgenic mouse model (h-a-synL62) and brain organoids derived from iPSCs of a PD patient. Wildtype mice that underwent daily restraint for 6 weeks presented typical chronic stress induced features, such as GR-deficiency and increased a-syn protein levels in the forebrain. In contrast, stress induced release of glucocorticoids had no effect on GR expression and normalized the level of a-syn in h-a-synL62 mice. Interestingly, these molecular alterations were reproduced in forebrain organoids generated from healthy and PD diseased donors after treatment with the synthetic glucocorticoid Dexamethasone (Dex) for 2 weeks. Since a-syn translation is regulated by iron, we analysed the expression of other proteins presenting iron response elements in control and treated organoids. On the transcriptional level, Dex treatment had similar effects on the expression of genes involved in iron homeostasis, however, protein levels of iron transporters and ferritin were differentially regulated in healthy and PD-derived organoids. Accordingly, IREB2, an iron response element binding protein that represses mRNA translation of a-syn, was upregulated in chronic Dex-treated PD but not healthy organoids. Together, our work provides a new link between a-syn overexpression, GR-deficiency and iron dyshomeostasis and their contribution to the development and progression of PD. Further, we established and validated a human 3D tissue culture model that can be used to study stress related diseases, offering replacement of research animals exposed to disturbing procedures.

Keywords: synuclein, brain organoid, iron

Poster: 991

CAPTURING DIFFERENT DISEASE SEVERITIES OF LIS1-LISSENCEPHALY IN HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS

Rossetti, Andrea Carlo[1], Krefft, Olivia[1], Maillard, Camille[2], Jabali, Ammar[3], Marsoner, Fabio[1], Wilkens, Ruven[1], Hoffrichter, Anne[1], Bahi-Buisson, Nadia[2], Koch, Philipp[1], Ladewig, Julia[1]

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Lissencephaly is a malformation of cortical development which is characterized by a smooth brain and a disorganized cortex. Heterozygous mutations in the LIS1 gene, encoding a microtubule-associated protein, were identified to cause lissencephaly with different severities. A direct correlation between a specific LIS1 mutation and the resulting severity grade of the patient could, however, so far not be drawn. We used pluripotent stem cell (PSC)-derived three-dimensional (3D) forebrain-type cerebral organoids, which faithfully recapitulate certain aspect of human brain development in vitro, to study disease severities of Lis1-lissencephaly. We found disease-related phenotypes in patient-specific organoids. More specifically, organoids derived from patients with mild, moderate or severe conditions reflect disease severity in the degree of alterations in cytoarchitecture and neurogenesis. In addition, we found a direct correlation in protein stability between mild versus severe phenotypes. Interestingly, phenotypic alterations can in part be reversed by stabilizing the microtubule array using the FDA approved drug EpothiloneD. Moreover, organoids from individuals with severe but not mild disorder exhibit impaired WNT signaling which can be partially rescued by GSK3ß inhibition. By that, our study demonstrates that organoid-based disease modeling is sensitive in recapitulating severity grade of the pathology and can be used to unravel human specific pathomechanisms.

Keywords: Neurodevelopment, Lis1-lissencephaly, Cerebral organoids

Poster: 992

DECIPHERING THE ROLE OF HUMAN-SPECIFIC GENES IN THE DEVELOPMENT OF THE HUMAN NEOCORTEX

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The human brain is remarkably complex, both structurally and functionally, and clearly represents the organ with the greatest progression during evolution. An expansion of progenitor cells in the human neocortex is thought to contribute to the increase in cortical size and complexity during evolution. The developmental mechanisms underlying the evolutionary changes are, however, poorly understood. With the advent of efficient gene editing technologies in human cells in combination with the ability to generate human pluripotent stem cells (PSC) and organotypic

brain organoids, we are now technologically equipped to decipher the molecular basis of the changes between our brain and that of our ancestors. We applied gene editing in PSCs and organoids derived thereof to study the function of ARHGAP11A and ARHGAP11B genes. While ARHGAP11A is a conserved gene through the primates' order, ARHGAP11B is human-specific. In the human neocortex, it plays an important role in increasing the basal radial glia cells pool. Using CRISPR/Cas9 mediated gene editing we generated double ARHGAP11A/B knockout lines. Following validation, we applied a standardized forebrain-type organoid protocol. When analysing the transgenic organoids and isogenic controls we could identify that ARHGAP11A/B impacts the expansion of early neuroepithelial (NES) cells and basal radial glial cells (bRGC). When investigating cleared transgenic organoids at day 15 we found enlarged NES structures. Further analysis revealed an increased expansion rate of the NES cells. Immunohistochemistry and single cell sequencing analysis on more mature organoids reveal an increased amount of bRGC in the transgenic cultures at day 55. Thus, our data indicate that transgenic organoids represent a powerful tool to map human-specific gene function in brain development, to correlate genetics to functional phenotypes and to complement the long tradition of knockout models in developmental biology and neuroscience.

Keywords: cerebral organoids, ARHGAP11B, basal radial glial cells

1:45 - 2:45 EDT

POSTER SESSION 10

CLINICAL APPLICATIONS

Poster: 821

COMPARISON OF SUBSTRATES FOR IN-VITRO HUMAN SMOOTH MUSCLE CELL DIFFERENTIATION FOR CLINICAL MANUFACTURING APPLICATIONS

Tran, Darlene[1], Wen, Yan2, Briggs, Mason[2], Ho, Dac Bang Tam[1], Gomes, Danielle[2], Villa, Miguel[1], Zhuang, Guobing[1], Shao, Qingchun[1], Sebastiano, Vittorio[1], Chen, Bertha[1]

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Pluripotent stem cell is a promising cell type for stem cell-derived therapies but there are significant challenges for their large-scale production, such as availability of cost-effective and clinical-grade reagents. The substrate that cells are cultured on affects cell proliferation and phenotype. Previous studies culturing human iPSC-derived smooth muscle cells (SMC) used gelatin or mouse-derived collagen IV, which presents immunogenic and infectious concerns for clinical applications. Thus, our goal was to evaluate clinically applicable substrates and their effect on the phenotype of human iPSC-derived SMC progenitor cells (pSMCs). iPSCs reprogrammed from human dermal fibroblasts and embryonic stem cells were differentiated into pSMCs as previously described. Extracellular matrix (ECM) adhesion optimization array was used to examine adherence of pSMCs to collagen, fibronectin, laminin, and vitronectin substrates. pSMCs were then cultured on varying concentrations and combinations of human placenta-derived collagen IV (hPDC IV), human fibroblast-derived collagen IV (hFDC IV), recombinant vitronectin (RV), and human-derived fibronectin (hDF) to assess cell adherence and proliferation. pSMCs cultured to passage 4 (P4) and terminally differentiated smooth muscle cells (tSMCs) at P5 were evaluated for pluripotency and SMC markers by q-PCR (OCT4, SOX2, Nanog, LIN28, SM22, αSMA, SMT, CNN1, MCH11, Elastin) and flow cytometry (CD30, CD31, CD34, αSMA, TRA-1-60, TRA-1-81, SMT). Contractility of tSMCs was accessed by carbachol assay. ECM array confirmed high pSMC adhesion to collagen and fibronectin. In culture, pSMCs adhered to and proliferated best in hPDC IV alone (5 µg/cm2) and a combination of hPDC IV (5 µg/cm2) and hDF (5 µg/cm2). We compared the phenotypes of the pSMCs cultured from the above substrates versus the commonly used mouse-derived collagen IV. pSMCs cultured on hPDC IV displayed similar levels of pluripotency markers, higher levels of certain SMC markers (SM22, SMT, CNN1, HCM11), and similar contractility. These data suggest that human placenta-derived collagen IV may be a viable substrate for clinical manufacturing of iPSC-derived smooth muscle cell therapies. Further studies are needed to assess in-vivo efficacy and safety of iPSC-derived SMCs cultured on hPDC IV.

Funding Source: This project was funded by the California Institute for Regenerative Medicine (CIRM) TRAN1-10958, PI B.Chen.

Keywords: Smooth Muscle Cell Differentiation, Smooth Muscle Cell Culture Substrate, iPSC-Derived Progenitor Smooth Muscle Cells

Poster: 825

STRATEGIES TO IMPROVE VIABILITY OF OTIC NEURONAL PROGENITOR SPHEROIDS DERIVED FROM HUMAN-INDUCED PLURIPOTENT STEM CELLS

Abboud, Yousef, Ghiringhelli, Matteo, Gepstein, Amira, Arbel, Gil, Huber, Irit, Gepstein, Lior

Department of Otolaryngology and Head and Neck Surgery - Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

Our group has recently demonstrated that arrangement of stem cell-derived otic neural progenitors (ONP) in three-dimensional spheroids leads to their improved survival after transplantation to the harsh cochlear microenvironment – a promising therapeutic approach for regenerating inner ear neurons to treat sensorineural hearing loss (SNHL). However, cell death in the spheroid core due to deprivation of nutrients and oxygen can limit such practice. We are currently investigating two strategies to solve this issue: 1) to integrate a perfusable vascular network derived from human umbilical vein endothelial cells (HUVEC) within the ONP spheroid and 2) to seed ONPs on a novel three-dimensional hydrogel organoid scaffold which allows nutrient and oxygen diffusion. More specifically, the first strategy leverages a microfluidic cell culture platform that is initially seeded with HUVECs, which are exposed to growth factors to induce angiogenic sprouting. Then, ONP spheroids are placed onto this generated vasculature. The ONPs used in both strategies were derived from human-induced pluripotent stem cells, following our already established protocol, and our data indicate that the resulting spheroids display the phenotypic and electrophysiological traits typical of neuronal progenitors. The first strategy resulted in generation of vessel-like structures capable of transporting medium and of surrounding and

pervading the ONP spheroids. The second strategy resulted in viable structures without the need for a vascular network. The results in the present work indicate a way to improve ONP viability in the inner ear, optimizing cell replacement therapy for SNHL. Therefore, in the next steps, these engineered structures will be transplanted into murine cochleae for survival assessment.

Keywords: Otic progenitors, Spheroids, Microfluidic perfusion culture

Poster: 827

IMPROVED OTIC NEURAL DIFFERENTIATION USING A MICROFLUIDICS-BASED 3D CELL CULTURE PLATFORM

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The reliable generation of spiral ganglion neurons (SGNs) from stem cells is fundamental to the development of cell-replacement therapies for sensorineural hearing loss. We have previously developed a differentiation protocol capable of generating otic neuronal progenitors (ONPs) and subsequently SGN-like cells from undifferentiated stem cell lines. However, this protocol requires prolonged time in culture and contemporaneously leads to the development of vestibular neuron (VN)-like cells from ONPs. We hypothesize that adjusting the concentrations and durations of exposure to signaling molecules Wnt and Sonic Hedgehog (Shh), which comodulate dorsoventral regionalization, and promoting the accumulation of endogenous factors will improve otic neural differentiation. To study these hypotheses, we developed a microfluidic device that can accommodate three-dimensional (3D) cell aggregates. Gradient modeling was performed to quantitatively assess fluid distribution within the microfluidic channel and ensure that each channel could be utilized as an independent treatment condition. To generate an artificial stem cell niche in vitro, microfluidic channels were filled with 0.30% nanofibrillar cellulose hydrogel (GrowDex-T, UPM-Kymmene, Helsinki, Finland). Hydrogel-embedded ONP spheroids generated according to our pre-established protocol were subjected to a range of Wnt and Shh concentrations and durations of exposure. The results indicate that by optimizing signaling molecule exposure and promoting the accumulation of endogenous factors, ONP differentiation toward the SGN phenotype was improved. Through further refinement of our protocol, we continue to advance the process of establishing clinically relevant models and treatments for sensorineural hearing loss.

Keywords: Inner ear regeneration, Otic progenitors, Microfluidics

Poster: 1001

IPSC-DERIVED NEOANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTE THERAPY FOR EWING SARCOMA

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The prognosis of Ewing sarcoma caused by EWS/FLI1 fusion is poor, especially after metastasis. The altered amino acid sequence at the break / fusion point of EWS/FLI1 when presented as a neoantigen evokes an immune response that targets EWS/FLI1+ sarcoma. Although therapy with cytotoxic T lymphocytes (CTL) targeted against altered EWS/FLI1 sequences at the gene break / fusion site may be effective, CTLs generated from peripheral blood are often exhausted because of continuous exposure to tumor antigens. We addressed this by generating iPSC-derived rejuvenated CTLs (rejTs) directed against the neoantigen encoded by the EWS/FLI1 fusion gene. RejTs have the same antigen-specificity as that of original CTLs, with higher proliferative capacity; they are functionally rejuvenated (Nishimura et al. Cell Stem Cell 2013, Ando et al. Stem Cell Reports 2015, Ando et al. Haematologica 2019, Honda et al. Molecular Therapy 2020). In this study, T-iPSCs established from an EWS/FLI1-specific CTLs differentiated into EWS/FLI1-specific rejTs. We first demonstrated the antigen-specific cytotoxicity of EWS/FLI1-rejTs against Ewing sarcoma in vitro. We then evaluated the antitumor effect of EWS/FLI1-rejTs against Ewing sarcoma in vivo to assess survival advantage. The mice were engrafted with luciferase labeled Ewing sarcoma cell line and treated with either EWS/FLI1-rejTs or original EWS/FLI1-bulk CTLs. The tumor growth was suppressed only in the mice treated with EWS/FLI1-rejTs. During 60 days of observation, EWS/FLI1-rejTs significantly prolonged survival (18-60 days) compared to untreated mice (p=0.01, 13-25 days) and EWS/FLI1-bulk CTLs (p=0.02, 22-35 days). Our results suggest that functionally rejuvenated EWS/FLI1-rejTs will be a very valuable novel treatment for Ewing sarcoma. The extremely strong potential advantage of EWS/FLI1-rejT therapy is the infinite generation of therapeutic CTLs from T-iPSCs. From this, EWS/FLI1-rejTs targeting the neoantigen would be a promising therapy for recurrent and metastatic cases of Ewing sarcoma, as well as for inoperable patients in the localized stage.

Keywords: neoantigen, rejuvenated CTL, Ewing sarcoma

Poster: 1002

HUMAN TROPHOBLAST CELL DERIVED EXTRACELLULAR VESICLES IMPROVE CELL THERAPY FOR MYOCARDIAL REPAIR

Abboud, Yousef, Ghiringhelli, Matteo, Gepstein, Amira, Huber, Irit, Gepstein, Lior

Physiology, Sohnis Research Laboratory for Cardiac Electrophysiology and Regenerative Medicine, the Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Haifa, Israel

The adult heart has limited regenerative capacity and therefore any significant cell loss may result in the development of progressive heart failure. Cardiomyocyte cell replacement therapy has become an option for myocardial repair but has been hampered by the significant donor cell loss after cell/tissue engraftment. Here, we examine the ability of trophoblast-cell-secreted extracellular vesicles (EVs) to improve cell-graft survival and myocardial function. EVs secreted by human trophoblast cells were collected by ultracentrifugations and characterized by a nanoparticle tracking analyzer. Their pro-survival and pro-angiogenic effects were initially evaluated by in-vitro assays, and their cargo was determined by a protein array. These in vitro assays confirmed the ability of human VT-EVs to improve cardiomyocyte survival and endothelial ability to form tubes. Myocardial infarction (MI) was then induced through permanent LAD coronary ligation in rats. Animals were randomized 8 days later to be transplanted with either human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), human villous trophoblast derived extracellular vesicles (HVT-EVs), both, or saline as a control. Cell engraftment and survival was evaluated by detailed histological evaluation. Myocardial function was evaluated through serial echocardiograms up to 30 days after transplantation. Graft-host coupling was assessed using optical mapping in the Langendorf isolated heart model by tracking the activity of a genetically-encoded voltage indicator expressed by the transplanted cells. In vivo, transplanting hiPSC-CMs, HVT-EVs or both limited left ventricular (LV) dilation and function deterioration compared to the control group. Furthermore, the functional outcome of the groups that contained both hiPSC-CMs and HVT-EVs was superior to transplanting hiPSC-CMs alone. We determined that at least part of the effect elicited by HVT-EVs was due to reduced cell apoptosis rate and enhanced angiogenesis. Extracellular vesicles secreted by human trophoblast cells can help improve cardiac function after MI either alone or even better if coupled with hiPSC-CMs transplantation, by attenuating cell death, improving tissue vascularization and cultivating damaged tissues resulting in better reception of the cell graft.

Keywords: trophoblast extracellular vesicles, human induced pluripotent stem cell, Myocardial infarction

Poster: 1003

IPSC CULTURE EXPANSION SELECTS AGAINST PUTATIVELY ACTIONABLE MUTATIONS IN THE MITOCHONDRIAL GENOME

Kosanke, Maike[1], Davenport, Colin[2], Szepes, Monika[1], Wiehlmann, Lutz[2], Kohrn, Tim[1], Dorda, Marie[2], Gruber, Jonas[1], Menge, Kaja[1], Sievert, Maike[1], Melchert, Anna[1], Gruh, Ina[1], Göhring, Gudrun[3], Martin, Ulrich[1]

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Therapeutic application of induced pluripotent stem cell (iPSC) derivatives requires comprehensive assessment of the integrity of their nuclear and mitochondrial DNA (mtDNA) to exclude oncogenic potential and functional deficits. iPSCs have been reported to harbor mtDNA variants at varying heteroplasmy levels. It is unknown, however, to which extent these genetic variants origin from their parental cells or from de novo mutagenesis, and whether dynamics in heteroplasmy levels are caused by inter- and intra-cellular selection or genetic drift. Sequencing of mtDNA of 26 iPSC clones did not reveal evidence for de novo mutagenesis, or for any selection processes during reprogramming or cardiomyocyte differentiation. Culture expansion, however, selected against putatively actionable mtDNA mutations and averts the mutational ratchet in iPSCs. Altogether, our results point towards a scenario in which intra-cellular selection of mtDNA variants during culture expansion shapes the mutational landscape of the mitochondrial genome. Mechanisms such as inter-cellular selection and genetic drift exert minor impact. Altogether, our results suggest that the bottleneck effect might have been overestimated, in context of the mtDNA genetic pool.

Keywords: Induced pluripotent stem cells, Mitochondrial genome, Genomic integrity

Poster: 1004

MITOCHONDRIAL SIRTUINS DISRUPT CELLULAR HOMEOSTASIS THROUGH THE PLURIPOTENCY AXIS

Jaiswal, Amit[1], Zhu, Xudong[2], Ju, Zhenyu[3]

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Sirtuins have been widely studied for their role in maintaining cellular homeostasis. They use the cofactor NAD+ for their activation. Three members of the sirtuin family, Sirt3-5 localize into mitochondria and regulate mitochondrial respiration, redox homeostasis and cellular metabolism. Previous studies have indicated that knockout of mitochondrial sirtuins interferes with the metabolic state of the cell. However, the collective role of mitochondrial sirtuins in cellular homeostasis of stem cells is yet to be investigated. In this study, we generated a novel triple knockout mouse embryonic stem cell line using CRISPR/Cas9 in order to investigate the effects of collective knockout of mitochondrial sirtuins. Our study revealed that deletion of mitochondrial sirtuins 3-5 disrupts the pluripotency axis as well as exhibited reduced differentiation potential. Furthermore, we also found that unavailability of mitochondrial sirtuins significantly increased ROS (reactive oxygen species) level accompanied by decreased levels of oxidative phosphorylation (OXPHOS) chain complexes in embryonic stem cells. In conclusion, we established a triple knockout embryonic stem cell model in order to decipher the collective effects of mitochondrial sirtuins and show for the first time that they can influence both mitochondrial homeostasis as well as stemness maintenance. Ongoing mechanistic studies will further enhance our understanding of sirtuin's cross-talk and would assist in unravelling their complex interplay in stem cells.

Keywords: CRISPR, Mitochondria, Embryonic Stem Cells

Poster: 1005

ADVANCED HUMAN PLURIPOTENT STEM CELL MANUFACTURING BY SEED TRAIN APPROACH AND UP-SCALING

Ullmann, Kevin, Manstein, Felix, Triebert, Wiebke, Teske, Jana, Lupanow, Victoria, Franke, Annika, Martin, Ulrich, Zweigerdt, Robert

Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), HTTG, Hannover Medical School, Hannover, Germany

Human pluripotent stem cell (hPSCs) derivatives have great potential for advanced drug screening, in vitro disease modelling and regenerative therapies. However, the envisioned routine application of these cells will require robust and economically viable production processes, compatible with industry and regulatory standards. Instrumented stirred tank bioreactors (STBR) are routinely applied for mammalian cell line cultivation in the biopharmaceutical industry. More recently, this platform has also been adapted to the matrix-free suspension culture of hPSCs by us and others. Manstein et al. have recently presented advanced high density hPSC bioprocessing in STBRs by metabolic control and in silico modelling. To close the gap between the research state and industry-compliance, we here demonstrate a seed train approach to ensure straightforward process upscaling. Chemical dissociation of the matrix-free cell-only hPSC aggregates from suspension culture crucially supported efficient single cell recovery, applied at each upscaling step. This strategy enabled uninterrupted maintenance of exponential cell growth over 5 passages for up to 20 days in suspension culture. Comparative analysis of differential passaging intervals revealed high process robustness and flexibility, opening the possibility for weekend-free cell production at highly controlled conditions. The strategy also enables minimal user interaction and fosters process automation. Importantly, unrestricted maintenance of hPSCs' pluripotency and differentiation potential was demonstrated by successful differentiation into functional derivatives, including efficient cardiomyogenesis. The presented novel strategy holds great potential for hPSC bioprocessing at large-scale paving the way towards the automated end-to-end production of hPS cells in closed systems, at clinically-relevant conditions and cell numbers.

Keywords: hPSC Bioprocessing, Seed Train, Scale-Up

Poster: 1006

CHARACTERISING THE GENOMIC INTEGRITY OF HUMAN EMBRYONIC STEM CELLS

Perfect, Leo, Raposo, Pedro, Konstantinidou, Chrysoula, Mattioli, Elisa, Holmes, Richard, Prince, Judith, Wise, Clare, Warre Cornish, Katherine, Hawkins, Ross, Abranches, Elsa

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Embryonic stem cells offer enormous promise as starting materials for advanced cell therapies. This is due to their defining properties of self-renewal and pluripotency. There are currently more than 70 clinical trials using pluripotent stem cell derivatives for a wide range of clinical presentations. In the future, their share of the cell therapy market is expected to increase as the field moves towards more scalable allogeneic approaches. At the UK Stem Cell Bank, we store and distribute all the human embryonic stem cells derived in the UK. This includes cell lines that were derived under the European Tissues and Cells Directive that can be made available for use as starting materials for cell therapies. One threat to their clinical exploitation is the observation that pluripotent stem cells are prone to acquire genetic changes in culture. Here, we report the genomic characterisation of 25 embryonic stem cell lines to examine their suitability for clinical applications. We have carried out whole genome sequencing, targeted oncogene sequencing and karyology. Rigorous control of cellular integrity, identity and purity is required to ensure the safety and efficacy of advanced cell therapies.

Keywords: ESC, genomic, stem cell

Poster: 1007

MICROENVIRONMENT PLAY A CRITICAL ROLE IN THE STRUCTURE AND FUNCTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED BETA CELLS

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People living with diabetes require frequent and painful insulin injections which makes life complicated. Despite technological advancements in glucose monitoring and close loop system, it is challenging to maintain normal glucose levels in the body leading to episodes of acute hyper- and hypo-glycemia; and consequently long-term debilitating secondary complications such as nephropathy, neuropathy, limb amputation, heart attack, stroke and death by a comma. Islet transplantation can make an individual insulin injection free for years, however is limited by the paucity of donors. Human pluripotent stem cell-derived beta cells provide a tangible alternative for cell replacement therapy. Despite comprehensive methods developed in the last six-years for beta-cell differentiation, the cells have failed to attain functional maturity and physiological insulin secretion. The native islet has a peculiar cytostructure where beta cells exist in close contact with other endocrine cells, vascular endothelial cells and endothelial secreted factors. Work since past 38-years suggests that inter- and intra-cellular interactions and the interaction of beta cells with basement membrane protein play a vital role in beta cell differentiation, proliferation, insulin gene synthesis and secretion. In the stem cell-derived spheroids beta cell niche has neither been investigated nor deliberately controlled until very recently. We have generated functionally active insulin secreting human pluripotent stem cell-derived beta cells in our lab. We have investigated beta cell niche in the differentiated spheroids and have demonstrated the ameliorating effect of extracellular matrix proteins on glucose stimulated insulin secretion in these cells. We are further analysing the effect of beta-cell niche on global gene expression pattern and thereby dissecting the underlying mechanism of favorably altered insulin secretion in stem cell-derived beta cells. This study will contribute significantly towards refining beta cell differentiation methods, disease modeling, drug screening and bringing us a step closer towards cell replacement therapy in people living with diabetes.

Funding Source: John and Ann Chong Fellowship (Philanthropic)

Keywords: Type 1 diabetes, Human pluripotent stem cell-derived beta cell, extracellular matrix

Poster: 1008

MESENCHYMAL STEM CELLS-DERIVED EXOSOMES LOADED WITH VITAMIN A AND QUERCETIN ALLEVIATE MICE ACUTE LIVER INJURY

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Acute liver injury is an acute inflammatory disease of the liver caused by a variety of factors. Severe liver injury can lead to serious complications and its treatment is extremely difficult. Mesenchymal stem cells- derived exosomes have been shown to have immunomodulatory effects and promote tissue repair, and are ideal drug delivery vehicles. Quercetin is a natural bioflavonoids widely found in fruits and vegetables and has anti-inflammatory and antioxidant effects. In this study, adipose mesenchymal stem cell-derived exosomes loaded with quercetin were administered intravenously to treat acute liver injury induced by CCl4 in mice. Exosomes greatly improved the solubility and stability of quercetin. To increase the liver targeting of exosomes, vitamin A was added to exosomes. The morphology of exosomes loaded with quercetin and vitamin A did not change greatly under electron microscope. The results showed that quercetin loaded exosomes could significantly reduce the serum AST and ALT levels of mice. Histopathological sections showed that quercetin loaded exosomes significantly alleviated hepatic lobule necrosis, and the effect was significantly better than that of exosomes alone. Exosomes loaded with vitamin A and quercetin provides a new hope for the treatment of severe acute liver injury.

Funding Source: National Natural Science Foundation of China (Grant No.32000511)

Keywords: Mesenchymal stem cells derived exosomes, Quercetin, Acute liver injury

Poster: 1009

SOFT THIOL-ENE CROSSLINKED ALGINATE HYDROGEL ENCAPSULATION REDUCES THE EXPRESSION OF ABERRANT EXTRACELLULAR MATRIX IN HUMAN KIDNEY ORGANOIDS

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Induced pluripotent stem cells differentiated to renal progenitors cultured in an air–liquid interface can self-organize into nephron-like structures. These organoids are a promising solution for the treatment of patients with kidney diseases. However, their maturity is limited, with a developmental plateau when cultured for longer period of time. Therefore, before these organoid can be translated to the clinic, these limitations must be overcome. Here we assessed the extracellular matrix (ECM) of the organoids to investigate the cause of the developmental plateau. We identified an increased aberrant ECM protein expression that indicated a possible fibrotic program when the organoids were cultured up to day 7+25 (7 days iPSC differentiation + 25 days air–liquid interface culture) in an air–liquid interface. We hypothesized a need for a three-dimensional environment to overcome this developmental plateau and aberrant ECM deposition as a better mimic of the environment around the developing kidney. Therefore, we selected a thiol-ene cross-linked alginate hydrogel, which mimics ECM fibrous morphology and mechanical properties. We synthesized a norbornene-functionalized alginate for thiol-ene crosslinking to form a soft (180 Pa) hydrogel. Kidney organoids at day 7+14 were encapsulated in this hydrogel and cultured for four subsequent days (day 7+18). The organoids encapsulated in the hydrogel maintained all major renal segments compared to the air–liquid interface control. Moreover, we observed a reduction of the proteins associated with fibrosis (e.g., type 1 collagen (COL1) and α-SMA) when organoids were encapsulated in the soft hydrogel for 4 days instead of the air–liquid interface. This work highlights the impact of hydrogel encapsulation on the ECM deposition and phenotype of kidney organoids.

Funding Source: This work is supported by the partners of Regenerative Medicine Crossing Borders (www.regmedxb.com). Powered by Health~Holland, Top Sector Life Sciences & Health.

Keywords: Kidney Organoids, ECM deposition, Biomaterials

Poster: 1010

CHARACTERIZING THE EFFECTS OF UTERUS-LIKE OXYGEN CONCENTRATIONS ON HUMAN KIDNEY ORGANOID MATURATION AND ENDOTHELIALISATION

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Organoids are gaining significant interest in the field of regenerative medicine. Their ability to self-organize from pluripotent stem cells into functional organ-like structures makes them candidates for organ replacement or repair. We aim to produce functional kidney organoids from induced pluripotent stem cells (iPSCs) to build an implantable kidney graft to reduce or replace dialysis for patients with end-stage kidney disease. For this, we differentiate iPSCs and subsequently form organoids by aggregation. After 18 days of culture, these are several millimeters in size and comprise small nephron-like structures, with tubular segments and an immature endothelium. We found that prolonged culture results in diminishing endothelial cells and deteriorating nephrons. Notably, the organoids grow in a transwell setup at an air-liquid interface, and are therefore directly exposed to a hyperoxic (21%) culture environment. This culture method has been used to culture kidney explants since the 1950s, however, there is little understanding of its effects. To gather more insight, we cultured our organoids in a hypoxic environment similar to the physiological range of oxygen in developing human kidneys. We investigated its effects on HIF1a/2a pathway activation and transcription of their target genes such as various VEGFa splice variants, EPO, ANG and GLUT1. We hypothesize that HIF pathway activation could lead to activation and enrichment of the endothelial cell population in the organoid. To confirm transcription of HIF target genes, we use common techniques such as qPCR and Westernblot as well as whole mount imaging facilitated by tissue clearing.

Ultimately, we aim to understand the effects of culturing kidney organoids in physiological oxygen concentrations for future transplantation.

Keywords: Kidney organoid, Physiological oxygen, Vascularization

Poster: 1011

SAFEGUARDING ETHICAL TREATMENT OF HUMAN SUBJECTS IN CLINICAL TRIALS

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During epidemics and pandemics, clinical trials are vitally important in determining whether innovative medical interventions are effective and safe, including vaccines, drugs, devices, surgical and radiological procedures, screening/diagnostic tests and stem cells. Human participants' use is also vital, as animal testing and computer simulation are not substitutes for testing people. Regulation of human participant research had its beginnings at a global level in response to World War II atrocities. In this current pandemic, scientists around the world are conducting clinical trials for a cure. It is tempting for scientists to compromise quality control and rigour in a desperate race to find a cure. But crises are not an excuse for lowering the bar of stringent ethical and scientific standards. Ethicists have warned scientists not to cave into bad science. Unethical experiments involving human participants is disturbing and reminds us of the past, e.g. the Tuskegee Study of Untreated Syphilis in the Negro Male. This presentation explores and critically analyses the current regulation on clinical trials and examines how it ensures all participants' ethical treatment in clinical trials. The best practice is recommended for adoption by scientists in other nations.

Keywords: Clinical trials, Ethics, Law

Poster: 1012

COMPARATIVE ANALYSIS OF STEM CELL THERAPY AND GENE THERAPY FOCUSING ON ARTICLES REGARDING HIGH IMPACT CLINICAL PHASE

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Over the past decade, stem cell therapy and gene therapy have been expected to be novel therapeutic modalities and have evolved and synergized with each other. However, from a translational aspect, stem cell therapy is overshadowed by gene therapy. Here, we defined "stem cell therapy" as the combination of PubMed's MeSH terms "Cell- and Tissue therapy" and "stem cell". We focused on the "high-impacted" papers and tried to find translational trend since the number of papers is almost the same for genetic therapy as it is for stem cell therapy. It was found that the number of articles with Impact Factor > 11.5 was almost the same with 528 articles for genetic therapy and 482 articles for Stem cell therapy. But in the clinical phase, it was found that the number of articles on stem cell therapy was less than the number of articles on gene therapy. However, when comparing the number of total articles in the clinical phase without taking into consideration their Impact Factor, there was little difference. The number of articles for the clinical phase of stem cell therapy was not significantly less. So, what is the cause for the difference in enrollment numbers in the higher Impact Factors? Without narrowing down a search for higher Impact Factor, Box plot analysis for the annual transition of impact factor in articles in the clinical phase revealed that gene therapy has more articles in the clinical phase of higher impact factor than stem cell therapy. One of the reasons for this is that there are many later clinical phase products that apply Chimeric Antigen Receptor T cells (CAR-T) or Small interfering RNA (siRNA) in gene therapy, and those results tend to be published as high-impact papers. Compared to stem cell therapy, gene therapy has a history of more than a decade in advance centering on congenital diseases. However, it is expected that these two therapeutic modalities will be fused and developed in the near future.

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: Gene therapy, Stem cell therapy, Comparison, Clinical stage, Publication, Impact factor, Dissemination of results

Poster: 1013

STUDY OF THE DEVELOPMENT OF ECO-SYSTEM TO ACCELERATE THE SPRED OF REGENERATIVE MEDICINE

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Regenerative and cell therapy is a medical treatment in which a portion of a donor's tissue or cells is harvested, multiplied and differentiated in a test tube, or transplanted into a patient with added functions in an attempt to cure the disease. In Japan, the promotion of research on regenerative medicine has been strengthened since the early 2000s, creating the ground for the creation of iPS cells. In addition, as is well known, Japan enacted a law in 2013 to promote regenerative medicine, and has been promoting policies aimed at industrializing regenerative medicine as a nation by revising laws related to pharmaceutical approval. In Japan, regenerative medicine products have been approved for inclusion in the public insurance reimbursement system, but issues that need to be resolved in order to further promote regenerative medicine in society are becoming clearer, such as the problem of providing expensive products as insurance treatment. We will provide an overview of Japan's regenerative medicine industrialization "ecosystem" that has been formed under these policies, and provide a case study of the problems that need to be solved in order to develop regenerative medicine and cell therapy.

Funding Source: Grant of Japan Agency for Medical Research and Development (AMED), Science of Science, Technology and Innovation Policy (JST RISTEX), and a SECOM Science and Technology Foundation research grant.

Keywords: Regenerative Medicine in Japan, public insurance reimbursement system, Pharmaceutical and Medical Device Act

Poster: 1014

ETHICAL DISCUSSION ON PATIENT COOPERATION IN STEM CELL CLINICAL RESEARCH IN JAPAN: A QUALITATIVE ANALYSIS ON PATIENT ORGANIZATIONS FOR RARE DISEASE

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The purpose of this study is to conduct an exploratory case study and qualitative analysis of the challenges and difficulties faced by patient advocacy groups in Japan in cooperating with and participating in clinical research on stem cell-based regenerative medicine. In Japan, stem cell-based clinical and commercialization research has been promoted in recent years, particularly for iPS cells. Patients with rare diseases are mainly involved in these clinical studies, and rare disease patient organizations are increasingly cooperating in recruiting patients and raising awareness about research. In this study, we conducted a qualitative survey using semi-structured interviews with several rare disease patient organizations in Japan cooperating in clinical research on regenerative medicine using iPS cells and other stem cells. A qualitative coding analysis of the interview data revealed several structures. One is an issue related to the unique characteristics of stem cell research. Clinical stem cell clinical research tends to be biased toward particular groups of patients, and patients in the early phases of progression are more likely to be research participants. Although the primary objective of the first-in-human study of iPS cells (iPSC-FIH study) is to evaluate the safety, patients with rare diseases, for whom treatment options are limited, tend to perceive participation in the study as an option similar to treatment, even though they understand the nature of the study. The second issue relates to the socio-economic situation of patient organizations in Japan. In our preliminary survey, 76% of rare disease patient organizations' valid responses had no full-time staff and were supported by unpaid volunteers. The lack of financial and social resources, which is a prerequisite for patient involvement, is a burden for patient organizations. The need to provide educational programs for participation in clinical research, including public education programs to train Expert Patients, was also identified. These results suggest ethical issues that should be considered in policy to accelerate patient advocacy groups' participation in the process of patient engagement in stem cell-based clinical research.

Funding Source: This research was supported by AMED under Grant Number JP21XX1122333 and KAKEN under Grant Number 19K13877.

Keywords: Ethics and Public Policy, Induced Pluripotent Stem Cell (iPSC), Patient Involvement

Poster: 1015

REGULATORY PRIMING: WHAT MAKES A STEM CELL LINE SUITABLE FOR THERAPEUTIC USE?

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The number of clinical trials using human pluripotent stem cells (hPSCs) to manufacture a broad range of cells for therapy are ever increasing, with particular emphasis on cell replacement for degenerative disorders and damage loss. After decades of unremitting efforts and trials, human pluripotent stem cell derivatives are becoming dominant. In order to assure that hPSC-based products can progress efficiently into safe and effective therapies, it is crucial that key considerations from ethical, scientific and regulatory perspectives have been adequately addressed and well documented. Furthermore, new developments to enhance their properties, increase safety and reduce the risk of immunological rejection also necessitates consideration. To enable researchers to select appropriate hPSCs lines for translational research and the development of clinical trials, the Human Pluripotent Stem Cell Registry (hPSCreg®; www.hpscreg.eu) and the International Stem Cell Banking Initiative (ISCBI; www.iscbi.org) have collaborated to consider criteria for demonstrating the scientific and ethical suitability of individual hPSC lines to be used in clinical translation. Furthermore, they have proposed standards for the kinds of donor and cell line data that would need to be collated for implementation in hPSCreg®. In this presentation, we will address best practices for the assessment of data fields required to demonstrate that certain hPSCs lines, hereafter called "regulatory primed" cells, may be suitable for use in clinical trials. The presentation will specifically consider current data fields in hPSCreg® that are required for certification of cell lines for research use and identify the specific additional information that may be required by national regulators when hPSC-based products are considered for clinical trials and/or market approvals. This data will also relate to assure qualification, ethical provenance and traceability of crucial information, giving researchers and product developers alike confidence in their selection of manufacturing cell substrates.

Keywords: hPSC-derived cell therapy, regulatory framework, data standards

Poster: 1016

TRANSPLANTATION OF IPSC-DERIVED CORNEAL ENDOTHELIAL SUBSTITUTES IN A MONKEY BULLOUS KERATOPATHY MODEL

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In order to provide regenerative therapy for millions of patients suffering from corneal blindness globally, we derived corneal endothelial cell substitute (CECSi) cells from induced pluripotent

stem cells (iPSCs) to treat corneal endothelial dysfunction (bullous keratopathy). We developed an efficient xeno-free protocol to mass-produce CECSi cells from both research grade (Ff-MH09s01 and Ff-I01s04) and clinical grade (QHJI01s04) iPSCs. CECSi cells formed a hexagonal confluent monolayer with Na, K-ATPase alpha 1 subunit expression (ATP1A1), tight junctions, N-cadherin adherence junction formation, and nuclear PITX2 expression, which are all characteristics of corneal endothelial cells. CECSi cells can be cryopreserved, and thawed CECSi cell suspensions also expressed N-cadherin and ATP1A1. Residual undifferentiated iPSCs in QHJI01s04-derived CECSi cells was below 0.01%. Frozen stocks of Ff-I01s04- and QHJI01s04-derived CECSi cells were transported, thawed and transplanted into a monkey bullous keratopathy model. CECSi-transplanted eyes significantly reduced corneal edema compared to control group. Our results may show a promising approach to provide bullous keratopathy patients with an iPS-cell-based regenerative cell therapy to recover useful vision.

Keywords: Cell therapy, Bullous keratopathy, iPS cells

Poster: 1017

USING CRISPR ACTIVATION FOR CELL REPROGRAMMING TO GENERATE PHOTORECEPTORS

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Recent advances in the field of cell reprogramming have enabled the use of master transcription factors to directly reprogram one somatic cell type to another without going through an intermediate stem cell state. This direct reprogramming technology can potentially be used to regenerate photoreceptor loss due to degenerative eye diseases. Photoreceptor degeneration is a common hallmark of many retinal diseases which can give rise to profound vision loss. As there is no effective treatment available to restore vision due to photoreceptor loss, the development of a reprogramming technology to convert Müller glial (MG) cells into photoreceptors represents a promising approach for photoreceptor regeneration. This study aims to develop a novel approach to generate photoreceptors in vitro, by reprogramming MG into induced rod photoreceptors (iRods). We optimised a CRISPR activation (CRISPRa) system which can be used to activate reprogramming factors in a multiplex manner in MG cells. Using this CRISPRa system, we performed screening and identified transcription factor cocktails that promote reprogramming of MG cells into iRods. RT-qPCR and immunocytochemistry results demonstrated the activation of photoreceptor markers in iRods. Also, multi-electrode array analysis showed that the iRods possess functional electrophysiology. Finally, we performed single cell transcriptome analysis to profile the iRods and compared with adult rod photoreceptors as a benchmark. Our results highlighted that iRod reprogramming induced activation of a panel of photoreceptor markers and promoted transcriptomic transitions towards photoreceptors. In summary, our study demonstrated the use of CRISPRa for direct reprogramming of MG to generate iRods. Successful generation of iRods in vitro will provide a platform for drug screening, disease modelling, and the application of gene therapy to treat a range of retinal diseases. This reprogramming method also has the potential to be applied in vivo in the future, offering an innovative approach to regenerate photoreceptors and alleviate blindness caused by retinal diseases.

Keywords: cell reprogramming, eye diseases, CRISPR

Poster: 1019

EFFICIENT PRODUCTION OF NEUTROPHILS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS THAT PREVENT MURINE LETHAL INFECTION WITH IMMUNE CELL RECRUITMENT

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Patients with granulocytopenia due to intensive chemotherapy are highly susceptible to severe infections. Although granulocyte transfusion therapy (GTX) is the promising therapeutic option besides antibiotics, its physical burden on donors is the main obstacle. Neutrophils derived from induced pluripotent stem cells (iPSCs) may become the alternative source for GTX. We have previously reported the establishment of expandable neutrophil progenitors (NeuPs) from human iPSCs by inducible expression of c-Myc and BMI1. Although NeuPs stopped expansion after 12 weeks, NeuPs differentiated into mature neutrophil-like cells (NeuCs) four days after silencing c-Myc and BMI1. In current study, we sophisticated culture method and achieved feeder-free and robust production enough to clinical application. To enhance the expandability, we focused on several BCL-2 family genes: BCL2A1, MCL1 and BCL-XL. The overexpression of each gene prolonged expansion for more than 16 weeks. Notably, BCL-XL enabled not only expansion for at least 24 weeks, but also feeder-free expansion, which is preferable to clinical application. To investigate the function, first we performed transcriptome analysis. Gene expression profiling of NeuCs revealed the enrichment of normal mature neutrophil-specific gene set. Gene ontology enrichment analysis confirmed the enrichment of inflammatory response with LPS. Second, we analyzed neutrophil function in vitro and confirmed that NeuCs acquired adhesion, migration, phagocytosis, and bactericidal function. Third, to assess the function in vivo, we performed in vivo imaging of NeuCs expressing luciferase in mouse model, which revealed that injected NeuCs accumulated specifically at the infection site. When S. aureus was simultaneously injected with NeuCs in mice intraperitoneally, NeuCs completely rescued the lethality. We also confirmed that activated NeuCs produced several cytokines and chemokines, and recruited murine immune cells such as macrophages and dendritic cells into inflammatory site. These data suggest that NeuCs have the migration and bactericidal capacity, and recruit immune cells to improve immune response in vivo. In summary, we achieved the feeder-free and prompt mass production of functional neutrophils, which can pave the way for donor-free GTX.

Keywords: iPSC-derived neutrophil, granulocyte transfusion therapy, expandable neutrophil progenitor

Poster: 1020

UPSCALING OF EX VIVO PRODUCTION OF RED BLOOD CELLS FOR TRANSFUSION PURPOSES

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Transfusion of donor-derived red blood cells (RBCs) is the most common form of cell therapy. Nevertheless, it faces challenges such as emerging blood-borne diseases, and supply limitations; for instance, in low-income countries, or for chronically transfused patients requiring special blood groups. Production of cultured RBCs (cRBCs) may be a potential alternative. Erythroid precursors can be cultured from hematopoietic progenitors, and differentiated into transfusion-ready cRBCs. The large number of cRBCs required for a single transfusion unit requires major innovations in the culture process. Our aim is to scale-up production, and to increase the cell density during expansion of erythroblast cultures and their subsequent differentiation to cRBCs. We optimized a protocol to expand erythroblast cultures using 0.5 and 3.0 L single-use stirred bioreactors. Erythroblasts produced in this system showed the same proliferation potential as those cultured in conventional static conditions. Oxygen availability appeared a critical process parameter for scale-up. Erythroblasts could be cultured at oxygen concentrations as low as 0.7 mg/L, controlled by intermittent sparging. Maximum shear stress at the impeller tip, predicted to be ~1.8 Pa, was mimicked in an orbital shaker setup to evaluate the effect of mixing on the culture. This level of shear stress induced Ca2+-dependent signaling pathways, including the calcineurin/NFAT pathway, and PKC-enhanced STAT5 signaling. As a result, erythroid maturation under differentiation conditions is enhanced by this level of shear stress. Cell erythroblast densities of up to 10 x 106 cells/mL were reached upon continuous media perfusion, 5X higher compared to the current expansion culture systems. Erythroblasts cultured at high density with perfusion maintained high viability and low spontaneous differentiation. This perfusion strategy leads to smaller culture volumes, although we currently use the same medium volume that would be used in stationary cultures. To reduce medium requirements, we study cell metabolism to assess which nutrients are limiting, and how we can supplement specific nutrients during perfusion. Changes in operating conditions of the stirred bioreactors or in medium composition will be tested on cell viability and differentiation potential.

Keywords: red blood cells bioreactor, erythroblast, transfusion medicine

Poster: 1021

ENGINEERING CLINICALLY RELEVANT BONE MARROW NICHES: THE RECIPROCAL CROSSTALK BETWEEN MESENCHYMAL AND HEMATOPOIETIC STEM CELLS MATTERS

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Transplantation therapies have driven the increasing demand to engineer artificial bone marrow niches to cultivate human mesenchymal stromal cells (MSCs) and hematopoietic stem and progenitor cells (HSPCs) in vitro. Therefore, tissue-engineering efforts currently focus on finding the right parameters to mimic the naïve bone marrow (BM) niches in order to tune MSCs and HSPC function. However, BM mimicry is still challenging due to its cellular and extracellular matrix complexity. An adequate in vitro BM model providing a predictive platform for basic science and the clinical translation is still lacking. Here, we developed a novel, xeno-free, MSC-based 3D BM model to mimic the transition zone between the BM's perivascular and osteoblastic niches. This allowed us to quantify how the reciprocal crosstalk between the two stem cells regulates their respective fate, i.e. via the extracellular matrix they produce and paracrine signaling. These insights have major implications for the engineering of naïve-like niches and their clinical applications.

Keywords: Bone Marrow Niche Engineering, Ex-vivo regulation of Hematopoietic Stem Cell, Mesenchymal Stromal Cell

Poster: 1022

THE STATE OF MESENCHYMAL STEM CELLS IN PATIENTS WITH TYPE 2 DIABETES AND THE PRESENCE OF NASH

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The aim of the study is to focus on mitochondrial dysfunction, in the context of NASH, mitochondrial function in stem cells is likely to be impaired. Mesenchymal stem cell separated of peripheral blood from diabetes 2 type (DT2) patients was collected in the context of a clinical protocol authorized by the local Ethics Committee of Ukraine Association of Bio-bank (Ukraine), with a license from the Ministry of Health of Ukraine 04/10/2018 No1813 and 27/03/2019 No1231 by the national competent authority for biobank cord blood, cell and, tissue therapy. ClinicalTrials.gov Identifier: NCT04642911. The study population (n = 96) was represented by diabetic patients from SI «ZIGUS NAMSU» in Kharkiv, Ukraine, and healthy volunteers. Patients were divided into two groups: group I consisted of patients with diabetes 2 type (DT2), group II - patients with DT2 complicated course of NASH (DT2 + NASH). The control group consisted of 25 conditionally healthy persons (men and women) of the same age. The health of patients strongly affects the status of MSCs. Those cells isolated from patients with type 2 diabetes (TD2) or from patients with TD2 accompanied by the NASH have the increased incidence of apoptosis, autophagy, accumulation of free radical molecules, and mitochondria deterioration. The mitochondrial membrane potential observed in these cells may be a protective mechanism that provides energy and building blocks to restore cellular homeosta-sis and control oxidative damage. Based on presented data, our conclusion is that crucial metabolic aspects of TD2 are indeed recapitulated at the systemic level and perspective therapeutic application of MSC iso-lated from TD2 patients may be limited due to their dysfunctionality.

Keywords: mesenchymal stem cells, type 2 diabetic, NASH

Poster: 1023

HUMAN IPSC-DERIVED MSCS AND THEIR TISSUE-DERIVED COUNTERPARTS: A COMPARITIVE ANALYSIS OF MSC HETEROGENITY

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Multipotent mesenchymal stromal cells (MSCs) are currently the subject of over 1200 clinical trials, with the capacity to treat a wide range of diseases due to their pro-regenerative, wound healing and immunomodulatory properties. However, they are traditionally sourced from tissue donations, meaning that translation into an effective cell therapy is affected by a lack of available donors, donor-donor variation, and a requirement to substantially expand isolated cells before reaching clinical doses. To address this, MSCs have more recently been derived from induced pluripotent stem cells, which have the potential to provide a virtually limitless supply of off-the-shelf, donor independent, batch-to-batch consistent cell therapies. Clinical trials investigating iPSC-derived MSCs (iMSCs) have reported very favourable results, nevertheless, questions still exist about the comparability of iMSCs to their tissue-derived counterparts. This is further complicated by the inherent heterogeneity of tissue-derived MSCs and the wide variety of mechanisms by which they exert their therapeutic potential. To address this, we have extensively characterised both clinical and commercial grade iMSCs in parallel with tissue-derived MSCs, from multiple donors and both adult and postnatal tissue sources. We have characterised both donor and source-specific distinctions in proliferation, metabolism, morphology, and differentiation capacity. Specifically, iMSCs demonstrate a high metabolic and proliferative potential, and enhanced osteogenic differentiation capacity. Furthermore, we have explored differences in individual surface-antigen profiles, inflammatory licensing, immunomodulatory capacity and secretome. Here too, iMSCs compare favourably, demonstrating effective in vitro inflammatory licensing and increased secretion of immunomodulatory factors, including indoleamine 2,3-dioxygenase. Our results also suggest that iMSCs display considerably less intercellular heterogeneity as well as bypassing much of the donor driven inconsistencies observed in conventional, tissue-derived MSCs. Collectively, our data provide new insights into the source and extent of MSC heterogeneity as well as evidence that supports the use of iMSCs as a scalable source of off-the-shelf cell therapies.

Keywords: iMSCs, Heterogeneity, Characterization

Poster: 1024

WNT-BANDAGE FOR BONE REPAIR

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The maintenance of human skeletal stem cells (hSSCs) and their progeny in bone defects is a major challenge. Recently, we eningeered a transplantable bandage containing a three-dimensional Wnt-induced osteogenic tissue model (WIOTM). This bandage facilitates the long-term viability of hSSCs (8 weeks) and their progeny, and enables bone repair in an in vivo mouse model of critical-sized calvarial defects. The newly forming bone is structurally comparable to mature cortical bone and consists of human and murine cells. Furthermore, we show that the mechanism of WIOTM formation is governed by Wnt-mediated asymmetric cell division of hSSCs. Covalently immobilizing Wnts onto synthetic materials can polarize single dividing hSSCs, orient the spindle and simultaneously generate a Wnt-proximal hSSC and a differentiation-prone Wnt-distal cell. Our results provide insight into the regulation of human osteogenesis and represent a promising approach to deliver human osteogenic constructs that can survive in vivo and contribute to bone repair. Importantly, the technology of the Wnt-bandage can be applied to other tissues where stem cells are Wnt-responsive.

Keywords: Stem cell therapy, Wnt-bandage for tissue repair, asymmetric stem cell division

Poster: 1025

RECONSTRUCTION OF DAMAGED NEURAL CIRCUITS IN A MOUSE STROKE MODEL

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Cell transplantation therapy using pluripotent stem cells for stroke has received much attention recently. However, no report shows behavioral improvement due to cell engraftment and neural circuit reconstruction. In this study, we first created a photothrombotic cerebral infarction model using rose bengal to achieve this goal. This model is simple, easy to use, highly reproducible, and has a fixed range of cerebral infarction. Then we transplanted 4, 5, and 6-week-old cerebral organoids derived from human induced pluripotent stem cells into the brains of the animals with this model. The organoids were grafted and showed neural fiber extension. As expected, 4-week-old organoids resulted in larger grafts. Animals with rich neural fiber extension from the grafts showed faster behavioral improvement than other animals. Our results presented a proof-of-concept of cell transplantation therapy for cerebral infarction.

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

Keywords: cell therapy, cerebral infarction, transplantation

Poster: 1026

TROPHOBLAST GLYCOPROTEIN IS A NOVEL MARKER FOR SORTING OF VENTRAL MESENCEPHALIC DA PRECURSORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Successful cell therapy for Parkinson's disease (PD) requires large numbers of homogeneous ventral mesencephalic dopaminergic (vmDA) precursors. Enrichment of vmDA precursors via cell-sorting is required to ensure high safety and efficacy of the cell therapy. Here, using LMX1A-eGFP knock-in reporter human embryonic stem cells, we discovered a novel surface antigen, trophoblast glycoprotein (TPBG), which was preferentially expressed in vmDA precursors. TPBG-targeted cell sorting enriched FOXA2+LMX1A+ vmDA precursors and helped attain efficient behavioral recovery of rodent PD models with increased numbers of TH+, NURR1+, and PITX3+ vmDA neurons in the grafts. Additionally, fewer proliferating cells were detected in TPBG+ cell-derived grafts than in TPBG- cell-derived grafts. Our approach is an efficient way to obtain enriched bona fide vmDA precursors, which could open a new avenue for effective PD treatment.

Funding Source: This study was supported by the Korea Health Technology R&D Project Grant (HI18C0829) through the Korea Health Industry Development Institute, founded by the Ministry of Health & Welfare.

Keywords: Trophoblast glycoprotein, Novel dopaminergic cell marker, Purified dopaminergic precursors

Poster: 1027

HIPSC-DERIVED NSCS EFFECTIVELY PROMOTE THE FUNCTIONAL RECOVERY OF ACUTE SPINAL CORD INJUYR IN MICE

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Spinal cord injury (SCI) is a common disease that results in motor and sensory disorders and even lifelong paralysis. The transplantation of stem cells, such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), or subsequently generated stem/progenitor cells, is predicted to be a promising treatment for SCI. In this study, we aimed to investigate effect of human iPSC-derived neural stem cells (hiPSC-NSCs) and umbilical cord-derived MSCs (huMSCs) in a mouse model of acute SCI. Acute SCI mice model were established and were randomly treated as phosphate-buffered saline (PBS) (control group), repaired with 1×105 hiPSC-NSCs (NSC group), and 1×105 huMSCs (MSC group), respectively, in a total of 54 mice (n = 18 each). Hind limb motor function was evaluated in open-field tests using the Basso Mouse Scale (BMS) at days post-operation (dpo) 1, 3, 5 and 7 after spinal cord injury, and weekly thereafter. Spinal cord and serum samples were harvested at dpo 7, 14 and 21. Haematoxylin-eosin (H&E) staining and Masson staining were used to evaluate the morphological changes and fibrosis area. The differentiation of the transplanted cells in vivo was evaluated with immunohistochemical staining. The hiPSC-NSC-treated group presented a significantly smaller glial fibrillary acidic protein (GFAP) positive area than MSC-treated mice at all time points. Additionally, MSC-transplanted mice had a similar GFAP+ area to mice receiving PBS. At dpo14, the immunostained hiPSC-NSCs were positive for SRY-related high-mobility-group (HMG)-box protein-2 (SOX2). Furthermore, the transplanted hiPSC-NSCs differentiated into GFAP-positive astrocytes and beta-III tubulin-positive neurons, whereas the transplanted huMSCs differentiated into GFAP-positive astrocytes. In addition, hiPSC-NSC transplantation reduced fibrosis formation and the inflammation level. Compared with the control or huMSC transplanted group, the group with transplantation of hiPSC-NSCs exhibited significantly improved behaviours, particularly limb coordination. hiPSC-NSCs promote functional recovery in mice with acute SCI by replacing missing neurons and attenuating fibrosis, glial scar formation, and inflammation.

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Keywords: spinal cord injury, induced pluripotent stem cell, neural stem cell

Poster: 1028

DEVELOPMENT OF TRANSPLANTABLE CEREBRAL ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Ischemic stroke is a major cause of disability in elderly people. To treat stroke patients, we are attempting to reconstruct the injured cerebrospinal tract (CST) by using induced pluripotent stem (iPS) cell-derived cerebral organoids. We have successfully induced cerebral organoids by using feeder-cultured human embryonic stem (ES) cells and have confirmed that the organoids extend neural fibers to the CST in the brains of normal mice. We then started to develop a cell product using a clinical-grade feeder-free iPSC line. We modified the maintenance culture condition called "pre-conditioning" and found that it improved the differentiation efficiency. We are now conducting animal experiments to confirm the safety and efficacy of the cell products.

Keywords: Cerebral Organoid, human iPS cells, Cell transplantation

Poster: 1029

ROBUST & SCALABLE PROCESS ENABLES THE TRANSPLANTATION OF HPSC-DERIVED FUNCTIONAL MICROTISSUES COMPOSED OF DOPAMINERGIC NEURONS WITH BEST-IN-CLASS RESULTS IN PRECLINICAL MODEL OF PARKINSON'S DISEASE

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Despite the development of efficient protocols to differentiate human pluripotent stem cells (hPSC) into dopaminergic neurons, the transplantation of mature DA neurons to treat Parkinson's disease is generally considered as a non-viable option. In mature neurons cultured in vitro, neurite outgrowth indeed greatly complexifies cell harvest and transplantation. To circumvent this issue, most cell replacement therapies in development for Parkinson's disease use dopaminergic progenitors. However, the clinical use of these multipotent cells remains challenging with

regards to : i) the in situ control over the graft's cellular identity and ii) the management of cell proliferation risks. In addition, such progenitor-based cell therapies rely on differentiation protocols which do not meet industrial requirements for scalable manufacturing in bioreactors. Since 2018, we have been addressing some of these challenges with the C-Stem™ technology. Using high-speed cell encapsulation microfluidics, hiPSC were grown in alginate shells and differentiated into functional microtissues composed of dopaminergic neurons. Following capsule dissolution, mature neural microtissues were transplanted into hemiparkinsonian rats, leading to full motor recovery within 8 weeks (vs 16 weeks with progenitors). We believe the reduction of time-to-effect is partly linked with the transplantation of mature neurons, which eliminates the time lag required for progenitor in situ differentiation. Importantly, we replicated our results with 3 different cell lines differentiated "in capsulo", demonstrating the robustness of the process. Capable of scaling-up hPSC production in industrial bioreactors (single batch of 15 billion hiPSC recently delivered in a 10L bioreactor), the C-Stem™ technology open new avenues for the production of large-scale commercial batches of dopaminergic neurons to treat Parkinson's disease. First-in-human trial with this novel therapeutic format is tentatively scheduled for 2024.

Keywords: Cell therapy, Parkinson's disease, Dopaminergic neurons

Poster: 1030

SAFETY AND EFFICACY OF FIRST-IN-HUMAN INTRATHECAL TRANSPLANTATION OF HUMAN ASTROCYTES (ASTRORX®) IN ALS PATIENTS: PHASE I/IIA CLINICAL TRIAL RESULTS

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AstroRx® is a cell-based therapy, composed of healthy and functional human astrocytes derived from embryonic stem cells. AstroRx® cells can protect neurons by several mechanisms of action that were demonstrated in in-vitro and in vivo preclinical studies. The clinical study hypothesis is that transplantation of astrocytes (AstroRx®) can compensate for the malfunctioning of endogenous astrocytes by restoring hampered physiological capabilities, i.e. reducing toxic compounds (e.g. reuptake of excessive glutamate), reducing oxidative stress, as well as by secreting multiple neurotrophic and neuroprotective factors. We conducted a Phase I/IIa, Open-Label, dose-escalating Clinical Study to Evaluate the Safety, Tolerability and Therapeutic Effects of Transplantation of AstroRx® in Patients with Amyotrophic Lateral Sclerosis (ALS). Enrolled patients were monitored for 3 months of "run-in period" to record their ALS progression. At the end of the run-in period, 5 patients were injected intrathecally with a single dose of 100*106 AstroRx® cells and 5 patients with a single dose of 250*106 cells. After treatment, the patients were monitored for additional 6 months for recording safety data and assessment of disease progression, as compared to the run-in period and for additional 6 months for long range safety. AstroRx® treatment was well tolerated in both doses and no treatment-related serious adverse events nor dose-limiting toxicities related to AstroRx® cells were reported. A potentially clinically meaningful decline in disease progression, assessed by the ALS Functional Rating Scale-Revised (ALSFRS-R), was observed during the first 3 months of the 6-month follow-up period in both cohorts. Single-dose transplantation of AstroRx® cells is safe and demonstrated a promising efficacy during the first 3 months. These results support a further, randomized-controlled, clinical trial with repeated doses of AstroRx® in patients with ALS, in order to prolong the time span of the clinical effect observed by the single dose.

Keywords: Astrocytes, Amyotrophic Lateral Sclerosis, Embryonic stem cells

Poster: 1031

HIGH THROUGHPUT SCREENING FOR DOPAMINERGIC NEURON-SPECIFIC TOXICITY IN HUMAN MIDBRAIN ORGANOIDS

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Three-dimensional (3D) cell culture systems, especially in the form of organ-like microtissues ("organoids"), have fueled hopes to develop the next generation of more physiologically relevant high throughput screens (HTS). However, the combination of complex 3D biology with the high degree of reproducibility and scale up required for HTS-applications has been challenging. We have recently developed a HTS-compatible, fully scalable workflow for the automated generation, maintenance, and high content analysis of human automated midbrain-like organoids (AMOs) in a standard 96-well format. The AMO's homogeneity makes them ideal candidates for compound screening campaigns. Here, we present the results of a proof-of-principle screen with a library of known neurotoxic and control compounds in a highly standardized turnkey model of the human midbrain. Moreover, we performed dose-response toxicity testing using selected compounds of interest to confirm the results of the primary screen. We quantified the impact of the compound treatment on both overall neural viability and midbrain dopaminergic neuron-specific toxicity in two cell lines via high content screening with single cell resolution. In conclusion, our results demonstrate screening for midbrain-specific toxicity in a physiologically-relevant organoid-based 3D human midbrain model. Our fully automated workflow can be easily adapted and scaled up for larger and more complex screening campaigns including not only toxicity evaluation but also disease models to identify novel therapeutic compounds, for example in Parkinson's Disease.

Funding Source: This work was funded by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No [669168]).

Keywords: Organoids, Midbrain, Screening

Poster: 1032

DEVELOPMENT OF A CLINICAL GRADE CELL PRODUCT BASED ON NEURAL STEM CELLS FROM CEREBROSPINAL FLUID

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Clinical-grade human neural stem cell (NSC) lines are usually obtained from the fetal central nervous system (CNS). Human NSC can also be isolated from the adult CNS in patients undergoing surgical procedures, or be derived from pluripotent stem cells and from somatic cells through reprogramming protocols. However, these procedures have ethical problems or are laborious, expensive, and commonly give rise to few NSC. We have recently discovered that NSC can be easily and robustly isolated from the cerebrospinal fluid (CSF) of preterm infants with intraventricular hemorrhage (IVH), an event that entails the rupture of the germinal zone, where NSC reside, into the ventricles. We are now carrying up the necessary experiments to develop a new cell product based on CSF-NSC. We have optimized CSF-NSC expansion by designing a specific coating based on the particular adhesion molecule expression profile of these cells. We have also optimized cell cryopreservation and intracranial delivery, finding that HypoThermosol® maintains CSF-NSC viable for 7 days and reduces the damage after passing through a needle. Comparative genomic hybridization and karyotyping analysis showed that CSF-NSC do not undergo transformation after long-term in vitro culture. CSF-NSC can be safely expanded, cryopreserved and transplanted into the brain of nude mice showing no sign of tumoral transformation. This cell product based on CSF-NSC could be useful for the development of new cell therapies, especially those aiming to attenuate the neurocognitive sequelae of IVH patients.

Funding Source: This work was supported by research funds from the Fundación Alicia Koplowitz and the Instituto de Salud Carlos III through the projects “DTS20/00108” and “PI19/00778”.

Keywords: NSC, Cell therapy, Intraventricular hemorrhage

Poster: 1033

PRELIMINAR ANALYSIS OF ENGINEERED FUNCTIONALLY ACTIVE HUMAN DERIVED CORTICAL NEUROSPHEROIDS FOR DRUG SCREENING AND PRECISION MEDICINE

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The continue development of differentiation protocols to generate human neural cells in vitro, allows more accurate investigations of the functional mechanisms arising in such complex networks, and generates great expectations for new treatments in neurodegenerative diseases for which effective therapies are not yet available. The use of 3D aggregates for neuropharmacological in vitro studies has shown great potentials and the advent of human patient specific in vitro models opens new avenues in the field of drug screening and precision medicine. Moreover, Neuronal Stem Cell (NSC) transplantation has the potential to revolutionize brain disease research, but still presents limitations that hamper the use in therapeutics. It has been shown how the injection of NSCs directly into the host, leads to a random integration into the tissue, while a targeted transplant is needed in the specific area affected by degeneration. An alternative approach would be to produce an already differentiated healthy 3D tissue, that shows all the functional and morphological features suitable for transplant into the degenerated area. To this end, we optimized a fast differentiation protocol to engineer excitatory cortical neurospheres with 1:1 ratio between neurons and astrocytes. We first evaluated its morphology by imaging and then we evaluated its functionality (i.e. electrophysiological activity) with glass-based 60 and CMOS-based 4096 micro-electrode arrays (MEAs). Our preliminary results show how the generated structures are viable and functionally active throughout their development. Furthermore, CMOS-MEAs revealed network properties that did not emerge from standard MEAs. Although the obtained results are preliminary, all neurospheroids adhered to substrates and developed functionally active neuritic arborizations, suggesting their efficient use for functional drugs screening applications and for future in vivo transplantation.

Funding Source: Co-financed by Programma Operativo Por FSE Regione Liguria 2014-2020, n. RLOF18ASSRIC

Keywords: brain-on-a-chip, human-induced pluripotent stem cells, micro-electrodes arrays

Poster: 1034

HUMAN PLACENTAL MESENCHYMAL STEM CELLS (MSCS) BOOST M2 ALVEOLAR OVER M1 BONE MARROW MACROPHAGES VIA IL-1BETA IN KLEBSIELLA-MEDIATED ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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Acute respiratory distress syndrome (ARDS) is a lethal complication of severe bacterial pneumonia due to over-exuberant immune responses during pathogen clearance, with Klebsiella pneumoniae (KP), a gram (-) bacteria with multi-drug resistance, among the most common cause. Both tissue-resident alveolar and bone marrow (BM)-recruited macrophage (MΦ) populations are involved and can be polarized to have divergent functions. Surprisingly, despite the known immunomodulatory properties of mesenchymal stem cells (MSCs), simultaneous interactions with resident-alveolar MΦs (AMΦs) and recruited-BMMΦs are largely unexplored. We assessed the therapeutic use of human placental-MSCs (PMSCs) in KP-ARDS, with the elucidation of the roles of AMΦs and BMMΦs. We developed a lethal pneumonia model using a clinically isolated KP strain of K2 serotype, with subsequent intravenous human PMSC treatment. AMΦ and BMMΦ analyses, histological evaluation, bacterial clearance, and mice survival were then assessed. To elucidate the role of pulmonary 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 recruited BMMΦ presence while preserving AMΦ numbers and enhancing their anti-bacterial functions, and an overall polarization of MΦs to an M2 phenotype. 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. Overall, M2 polarization and preferential rescue of AMΦs over BMMΦs by PMSC treatment were critical in improving KP-related ARDS outcomes.

Keywords: Acute respiratory distress syndrome (ARDS), placental-mesenchymal stem cells (PMSCs), alveolar macrophages (AMΦs)

Poster: 1035

RESIDENT VS. NON-RESIDENT HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELL INTERACTIONS WITH B LYMPHOCYTES RESULT IN DISPARATE OUTCOMES

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Multipotent human mesenchymal stromal cells (MSCs) from multiple organs including the bone marrow (BM) and placenta harbor clinically relevant immunomodulation best demonstrated towards T lymphocytes. Surprisingly, there is limited knowledge on interactions with B lymphocytes, which originate from the BM where there is a resident MSC. With increasing data demonstrating MSC tissue-specific propensities impacting therapeutic outcome, we therefore investigated the interactions of BM-MSCs—its resident and 'niche' MSC—and placental MSCs (P-MSC), another source of MSCs with well-characterized immunomodulatory properties, on the global functional outcomes of pan-peripheral B cell populations. We found that P-MSCs but not BM-MSCs significantly inhibit proliferation and further differentiation of stimulated human peripheral B populations in vitro. Moreover, while BM-MSCs preserve multiple IL-10-producing regulatory B cell (Breg) subsets, P-MSCs significantly increase all subsets. To corroborate these in vitro findings in vivo, we used a mouse model of B cell activation and found that adoptive transfer of P-MSCs but not BM-MSCs significantly decreased activated B220+ B cells. Moreover, adoptive transfer of P-MSCs but not BM-MSCs significantly decreased overall B220+ B cell proliferation and further differentiation, similar to the in vitro findings. P-MSCs also increased two populations of IL-10-producing murine Bregs more strongly than BM-MSCs. Transcriptome analyses demonstrated multifactorial differences between BM- and P-MSCs in the profile of relevant factors involved in B lymphocyte proliferation and differentiation. Our results highlight the divergent outcomes of tissue-specific MSCs interactions with peripheral B cells, and demonstrate the importance of understanding tissue-specific differences to achieve more efficacious outcome with MSC therapy.

Keywords: Human mesenchymal stromal cells (MSCs), bone marrow (BM), placenta, peripheral B lymphocytes, interleukin-10 (IL-10), regulatory B cells (Bregs), tissue specificity., Human mesenchymal stromal cells (MSCs), bone marrow (BM), placenta, peripheral B lymphocytes, interleukin-10 (IL-10), regulatory B cells (Bregs), tissue specificity., MSC, BM, placenta, B cell, tissue specificity