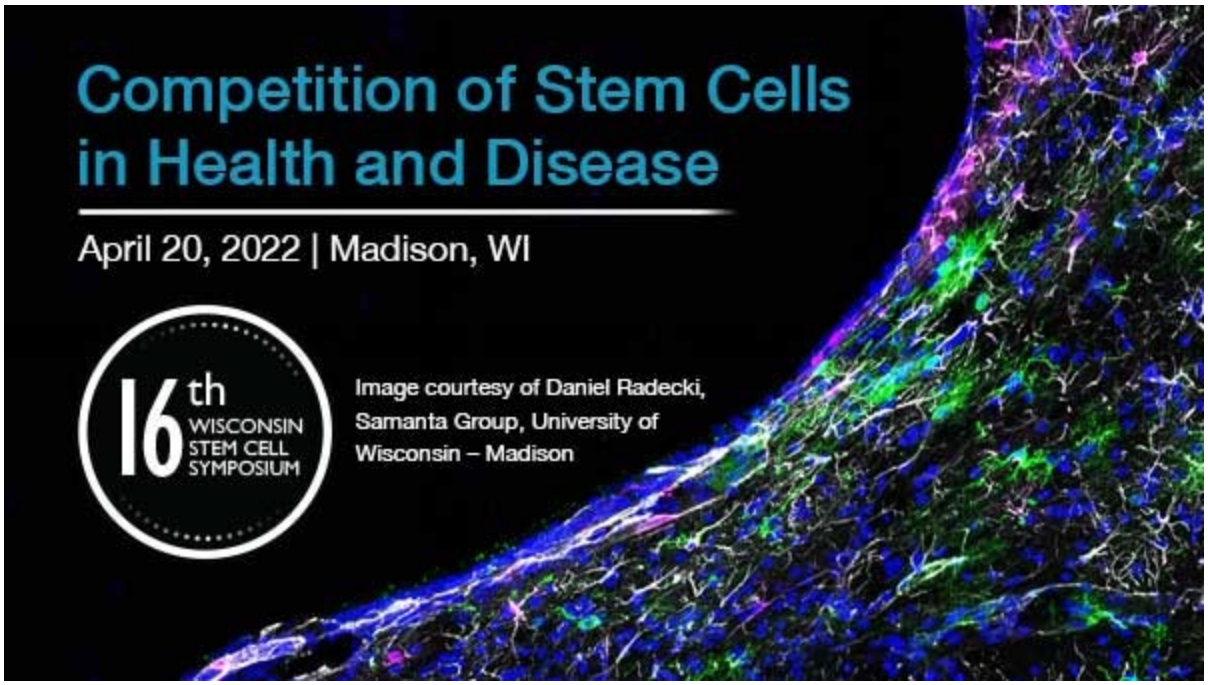

POSTER CONTEST FINALISTS

NOTE: Each submitter's name is in bold and italicized.

(1) An in vitro bone marrow niche to harbor blood stem cells for small molecule screening

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The bone marrow (BM) is a complex microenvironment that maintains and regulates hematopoietic stem/progenitor cells (HSPCs) throughout life. Steady-state HSPCs are regulated by an interdependent network of mesenchymal stromal cells (MSCs), nerve fibers, endothelial cells (ECs), and other hematopoietic cells. Understanding the interactions in these intricate niches will provide opportunities for HSPC-related therapies and immune modulation. We have assembled fluorescently labeled blue HSPCs, green MSCs, and red ECs in a serum-free co-culture system, thereby simulating aspects of the BM niche. We have adapted our co-culture system for high throughput imaging-based screening and developed an automated workflow to analyze HSPC interactions with BM niche cells. By measuring the distance of HSPCs to the closest MSCs, our data shows that the majority of HSPCs in vitro remain proximal to MSCs, as is observed in the endogenous niche. As proof-of-concept, we demonstrate that a drug used in the clinic to mobilize donor stem cells, the CXCR4 antagonist AMD3100, increases the distance of HSPCs from MSCs in our in vitro niche. We will use our in vitro screening platform to search for small molecules that can modulate HSPC interactions with the niche. We hope this will yield novel therapeutics for improvement of clinical HSPC transplantation.

(2) CTR9 drives osteochondral lineage differentiation of human mesenchymal stem/stromal cells (MSCs) via reciprocal regulation of EZH2-H3K27me3 and BMP-2 signaling

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Cell-fate determination of human mesenchymal stem/stromal cells (hMSCs) is finely regulated by lineage-specific transcription factors and epigenetic enzymes. Interestingly, we found that CTR9, a key scaffold subunit of Polymerase Associated Factor Complex (PAFc), selectively regulates hMSCs differentiation to osteoblasts and chondrocytes, but not to adipocytes. CTR9 knockdown in hMSCs strongly impaired osteogenic and chondrogenic differentiation as shown by the attenuated induction of Alkaline Phosphatase activity, decreased osteocalcin or aggrecan secretion and reduced formation of extracellular calcium or cartilage matrix, whereas adipocyte differentiation was not significantly affected. An in vivo ectopic osteogenesis assay confirmed the essentiality of CTR9 expression for hMSC-derived bone formation. The lineage-specific role of CTR9 is not due to modulation of stem cell characteristics of hMSCs. Instead, CTR9 counteracts the activity of EZH2, the epigenetic enzyme that deposits transcriptional repressive modification -- H3K27me3, a crucial histone mark of MSC lineage-specific differentiation. In accordance with the gain of H3K27me3 in CTR9 KD MSCs, the osteogenic differentiation defects of CTR9 KD hMSCs can be partially rescued by treatment with EZH2 inhibitors, supporting a CTR9-driven epigenetic mechanism in regulating MSC lineage differentiation. Transcriptome analysis further identify an early osteo-conductive gene Bone Morphology Protein-2 (BMP-2) as a potent downstream effector of CTR9 which further sustained by the observations of reduced BMP-2 secretion and its membrane anchorage as well as the interruption of BMP-SMAD intra-cellular signaling when CTR9 was lost. Supplement of recombinant BMP-2 peptide in the osteo-inductive medium is sufficient to overturn the osteogenic defects caused by CTR9 KD, proposing a central hub model of CTR9-H3K27me3-BMP-2 axis in fine-tuning osteogenesis governed by sequential and spatial epigenetic regulations.

(3) Posttranscriptional control of pluripotency by histone demethylases

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Pluripotent stem cells such as embryonic stem cells (ESCs) can self-renew indefinitely and differentiate into any cell type given the right stimulus. The plasticity of ESCs, is linked to a unique chromatin structure when compared to somatic cells, which is less compacted and depleted for histone modifications associated with gene repression, such H3K9 methylation. We have found that the H3K9me1/2 demethylases KDM3A and KDM3B have important roles in the acquisition of pluripotency. Although the double knockout (DKO) of *Kdm3a* and *Kdm3b* is lethal in both mice and ESCs, there are very few changes in steady state gene expression and the mechanism of gene regulation by these proteins remains unknown.
Therefore, we performed unbiased immunoaffinity purification of KDM3A or KDM3B followed by mass spectrometry to identify associated proteins. Surprisingly, KDM3A/3B were associated

with the RNA processing machinery rather than the expected heterochromatin proteins. We orthogonally validated the interaction of KDM3B with the splicing regulator PRMT5. To investigate if KDM3A/3B had a role in splicing we first generated KDM3B KO ESCs and knocked in a "degron" domain into KDM3A that allows for the rapid inducible degradation of the protein. We then performed whole transcriptome RNA sequencing after four hours of degradation and found misregulation of several canonical splicing pathways. Interestingly, the transcripts that retained exons in the KDM3A/3B KO had functions in chromatin and transcription regulation as well as response to DNA damage. Transcripts that lost exons were enriched for kinase function such as ATP binding. Thus, we have uncovered an unexpected function for histone demethylases in splicing selection of transcripts. We propose that this mechanism could be used to control pluripotency exit during the rapidly changing environment of a developing organism.

(4) HEMATOPOIETIC STEM AND PROGENITOR CELL DIFFERENTIATION INTO B CELLS IS REGULATED BY B CELL-PRODUCED GABA VIA A FEEDBACK LOOP

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Hematopoietic stem and progenitor cells (HSPCs) maintain blood production throughout life via self-renewal and multi-lineage differentiation. This makes HSPC transplantation a curative treatment for many hematopoietic diseases. In the bone marrow (BM) niche, many factors, including the nervous system, regulate HSPC behavior and function.Gamma Aminobutyric acid (GABA) receptor B subunit 1 (Gabbr1) is known to regulate HSPC proliferation and differentiation into B cells. We discovered that its ligand, GABA, is also produced in the BM by B cells. We hypothesized that GABA-producing B cells inthe BM can regulate their own production via a feedback loop with the Gabbr1 receptor on HSPCs. To test this, we tracked differentiation of HSPCs into B cells over time using the OP9 co-culture system. Using flow cytometry and liquid chromatography-mass spectrometry (LC-MS) of conditioned media, we measured HSPC differentiation and GABA production over time. We observed a dramatic increase in GABA levels in themedia, concurrent with an eleven-fold increase in B cells. Addition of Baclofen, aGABBR1 agonist, modulated HSPC differentiation towards a B cell fate. To further investigate the dynamics of B cell differentiation and GABA production, we used genetic tools to delete Gad1&2, the enzymes responsible for GABA production, specifically in B cells. We crossed Gad1/2-floxed mice to Mb1-Cre mice, deleting these enzymes from the earliest stages of B cell production. Differentiation of Gad-deficient HSPCs in cocultures produced significantly fewer B cells. We validated reduction of GABA levels during differentiation by LC-MS of culture media. Addition of GABBR1 agonist partially rescued loss of GABA production and promoted B cell production. Our data suggests that GABA produced by B cells stimulates Gabbr1 receptors on HSPCs to promote further differentiation.

(5) Acute Shear Stress and Piezo1 Activation Reduce Efflux Transport Activity in Blood-Brain Barrier in vitro Models

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The human brain vasculature uniquely regulates the influx of various nutrients into the brain parenchyma while restricting the brain penetration of toxins and blood constituents. Blood-brain barrier (BBB) properties include efflux transporter activity which combines with passive

barrier properties to limit the brain uptake of pharmaceuticals, creating a roadblock for neurotherapeutic development. Previous research has demonstrated that regional neuronal activation in a mouse model results in a local increase in permeability to a blood-borne P-glycoprotein (P-gp) substrate. We hypothesized that this increase in permeability may be partly driven by the transient increase in blood flow that accompanies neuronal activation. To test this hypothesis, we utilized human pluripotent stem cell (hPSC) and primary rat brain microvascular endothelial cell (BMEC) models of the BBB. We observed that 3 hours of 16 dyne/cm2 shear stress was sufficient to reduce P-gp efflux transport of the Rhodamine123 fluorescent substrate in primary rat BMECs. In addition, to identify a potential molecular mechanism of the shear-induced phenotype, we investigated the impact of pharmacological activation of Piezo1, a recently discovered mechanosensitive ion channel thought to be involved in vascular pathfinding and development, at the BBB. Application of the selective Piezo1 agonist, Yoda1, in our hPSC BMEC-like cell and primary rat BMEC models led to a reduction in P-gp activity after 3 hours of treatment, but not after 24 hours, suggesting a compensatory mechanism. Utilizing an hPSC line with a genetically encoded calcium indicator (GCaMP), we confirmed that application of the Piezo1 agonist led to an influx of calcium, a known modulator of P-gp activity, in the hPSC BBB model. Overall, this suggests that shear stress and Piezo1 may result in calcium mediated inhibition of efflux transport at the BBB, a potential mechanism for energy conservation within the brain vasculature during neuronal activation.

(6) **Generation of GD2-CAR neutrophils from hPSCs for targeted cancer immunotherapy of solid tumors**

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Chimeric antigen receptor (CAR) T cell and NK cell therapies already been successful in the eradication of lymphoid malignancies. CAR-lymphocytes are incapable to enter into the solid tumors and they also lose their activity within immunosuppressive tumor environment. Thus, opportunities exist for new immunotherapies for specific targeting of solid tumors using CAR-weaponized neutrophils which are capable of cytotoxicity and migration into solid tumors. However, generation of CAR neutrophils from peripheral blood represent a significant challenge due to their very short life-span. Human pluripotent stem cells (hPSCs) are a logical alternative for large-scale production of CAR neutrophils due to their renewability and uniform quality. In our study, we generated hPSCs with GD2 CARs integrated into *AAVS1* locus and differentiated into neutrophils using serum- and xeno-free differentiation system based on modified *ETV2* mRNA. Disialoganglioside GD2 antigen is highly expressed in neuroblastoma, glioma and melanoma cancer, and therefore a viable target for immunotherapy. Neutrophils generated from GD2 CAR iPSCs, as compared to wild type (WT), demonstrated superior cytotoxicity *in vitro* against $GD2^+$ WM266-4 melanoma and CHLA20 neuroblastoma, while no differences of cytotoxicity were observed against GD2-negative SKOV3 ovarian and SK-BR3 breast cancer cells, indicating the specificity of anti-tumor therapeutic effect of CAR neutrophils. To assess *in vivo* potential of GD2 CAR neutrophils, NCG mice were inoculated intraperitoneally (IP) with $3x10^5$ Luc2-$eGFP^+$ WM266-4 melanoma cells and engraftment was assessed by IVIS bioluminescent imaging. On day 4 post WM266-4 injection, mice were either treated with 10^7 WT or GD2 CAR

neutrophils via IP injection every 7 days. Upon assessment over 30 days, GD2 CAR neutrophil-treated mice showed reduced tumor burden compared to WT neutrophil-treated or untreated mice. Collectively, our studies demonstrate a feasibility of using hPSC-derived CAR-neutrophils for immunotherapies against solid tumor cancers.

(7) **Investigating the Regulatory Dynamics of Somatic Cell Reprogramming to Induced Pluripotent Stem Cells (iPSCs)**

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Induced pluripotent stem cells (iPSCs), are the favored tool for regenerative therapy because they are derived from somatic cells and forgo the practical and ethical barriers associated with embryonic stem cells. However, the efficiency of reprogramming cells to iPSCs is only ~5%. Using a rationally designed combination of small molecules that perturb the somatic epigenome to resemble that of pluripotent cells, along with signaling inhibitors, we increased mouse reprogramming to ~40% efficiency. Using single-cell RNA-sequencing, we discovered that, contrary to the dogma from previous population-based studies, the hallmarks of the transition to pluripotency, such as shutoff of somatic expression, gain of indefinite self-renewal, and activation of pluripotent gene regulatory networks, are independently acquired. Here, we applied the single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq) to investigate chromatin dynamics during the cell fate change to pluripotency. There were about 3-fold more accessible intergenic regions than those restricted to genic loci, suggesting a global epigenome rewiring. We find that accessibility of pluripotent loci precedes their expression and is followed by the opening of regions containing their motifs. The activity of the transcription factor Tcfap2c is essential for the transition to bona fide iPSCs that are independent of exogenous reprogramming factors. Finally, we have developed a new regression-based algorithm (scCISINT) which we used to identify a potential super enhancer that is transiently acquired during reprogramming. Current studies aim at validating the functional importance of this super enhancer using CRISPRi techniques. Altogether, our studies uncover important insights into the control of cell identity.

(8) TGFß1-Gpnmb signaling inhibits oligodendrogenesis from adult neural stem cells following demyelination.

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Demyelinating diseases, such as multiple sclerosis (MS), are debilitating diseases where the underlying degenerative and potential regenerative mechanisms are incompletely understood. Since regeneration of oligodendrocytes (OLs) and myelin can prevent neurodegeneration, understanding the molecular mechanisms of remyelination is essential to design therapeutic strategies. Adult neural stem cells (aNSCs) lining the lateral ventricles in the mammalian brain have the potential to regenerate myelin. In the healthy brain, a subset of these aNSCs express the transcription factor Gli1, migrate to the olfactory bulb and differentiate into interneurons and astrocytes, but not OLs. Following demyelination, these aNSCs can be recruited to lesions where they can differentiate into myelinating OLs. However, the pathways regulating aNSC recruitment, and their ability to generate OLs in adults is incompletely understood. Furthermore, remyelination is enhanced when Gli1 is inhibited in these aNSCs. By analyzing the transcriptome of these cells following demyelination, we identified GlycoProtein Non-metastatic

Melanoma B (Gpnmb) as the most significantly downregulated gene in aNSCs lacking Gli1 expression, suggesting its role as a negative regulator of remyelination. Indeed, global loss of Gpnmb in Gpnmb-LacZ knock-in mice, resulted in enhanced generation of OLs from Gli1 aNSCs following demyelination. Loss of Gpnmb also downregulated the expression of TGFßR2, a TGFß1 receptor subunit, which suggested that Gpnmb acts downstream or in tandem with TGFß1. Remarkably, in vitro treatment of aNSCs by TGFß1 lead to upregulation of Gpnmb. Overexpressing Gpnmb in aNSCs further lead to upregulation of TGFßR2 along with inhibition of mature OL generation in vitro. Consistently, genetic loss of TGFßR2 specifically in Gli1 aNSCs, resulted in enhanced recruitment and generation of mature OLs from aNSCs in lesions. Thus, TGFß1 released from demyelinating lesions, induces Gpnmb expression, which further upregulates TGFßR2 to amplify the pathway in Gli1 aNSCs, thereby inhibiting their recruitment and differentiation into mature OLs.

(9) Multi-omic assessment of the competitive landscape underlying variability in hPSC-cardiomyocyte differentiation outcomes

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Numerous protocols exist for differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes (CMs). Although these directed methods have improved in efficiency over the past decade, they remain highly variable in their resultant purities/efficiencies. This substantial heterogeneity is due to competition of numerous divergent pathways at play throughout the differentiation process, with significantly more off-target resultant cell types than the one on-target hPSC-CM. To better understand this temporal competitive landscape, and develop new methods to bias the specification to hPSC-CMs, we have undertaken a multi-omic discovery approach to identify key temporal differences in cell attributes between high- and low-purity hPSC-CM differentiations. Specifically, we are combining metabolomic, proteomic, lipidomic, and transcriptomic analyses collected throughout the differentiation process for high- and low-purity (as assessed by terminal %cTnT+ via flow cytometry) differentiation batches. These data are leveraged to provide dynamic, systems-level insights into underlying mechanisms which drive these populations to divergent endpoints and inform novel differentiation protocol perturbations.

To date we have begun to identify competitive pathways very early within the differentiation process, putatively giving rise to these divergent endpoints. These findings are allowing us to 1) identify novel markers for real-time monitoring to be able to continually predict final product outcomes (i.e. purity of the final hPSC-CM population), 2) develop new methods to provide a competitive advantage for our desired cell type (i.e. enhance hPSC-CM terminal purity), and 3) augment fundamental understanding of early developmental fate decisions.

(10) Generation of Anti-tumorigenic Macrophages from SIRPa-Knockout Human Pluripotent Stem Cells

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Human macrophages have diverse phenotypes and functions that can be harnessed for antitumor immunotherapy applications. Solid tumor cancers have been especially difficult to

treat with current immunotherapies due to the immunosuppressive tumor microenvironment (TME) which actively excludes T cells and polarizes immune cells into tumor-supporting players. Many cancers overexpress the “don’t eat me” cell surface receptor, CD47, which is recognized by SIRPa on macrophages and blocks phagocytosis of cancer cells. Human induced pluripotent stem cells (iPSCs) serve as a renewable and genetically pliable source to generate iPSC-Macrophages (iMacs) for the treatment of solid tumor cancers. Macrophages have unique advantages for targeting solid tumors including access to the hostile TME and natural capability for phagocytosis, cytotoxicity, and migration; making them an attractive candidate for immunotherapy. We generated SIRPa-knockout iPSCs and developed chemically defined serum- and feeder-free protocols for efficient generation of macrophages by 2D monolayer hemogenic endothelium formation, followed by differentiation into monocytic cells and M0 macrophages using M-CSF, IL-3, and IL-6. SIRPa-KO iMacs displayed a similar phenotype to Wildtype (WT) iMacs and polarize into M1 and M2 macrophages when activated by LPS+IFN-gamma or IL-4, respectively. During an *in vitro* cancer challenge, SIRPa-KO iMacs have superior phagocytic and cytotoxic capabilities of multiple CD47+ cancer cell lines with addition of cancer-specific monoclonal antibodies (mAb) than the WT counterpart. We observed little to no phagocytosis or killing of CD47+ cancer cells by SIRPa-KO iMacs without mAb, substantiating the safety of SIRPa-KO iMacs interacting with healthy CD47+ cells in a clinical context. In this novel system, we conclude that *in vitro* hematopoiesis is unaffected by genetically knocking-out SIRPa in iPSCs and subsequent SIRPa-KO iMacs are functional effector immune cells that confer superior antitumor potential against CD47+ solid tumor cancers and may be clinically translated into an immunotherapy.

(11) Sarcopenia on a dish: myogenic progenitors derived from human pluripotent stem cells

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There is currently no effective treatment for sarcopenia, the degenerative loss of skeletal muscle mass and function associated with natural aging. Age-specific effects of human serum or plasma on skeletal muscle cells have been demonstrated in several studies using primary cultures, but these models have limited availability and reproducibility due to the scarcity, sample-dependent heterogeneity, and rapid senescence of primary cells. To explore the ability to use human pluripotent stem cells (hPSCs) as an unlimited cell source for an *in vitro* model of muscle aging, we derived myogenic progenitors using our published transgene-free and serum-free floating sphere culture method. Previously, we established a model of sarcopenia using hPSC-derived myogenic progenitors supplemented with aged rat sera. We replicated our work in this study by replacing rat sera with human plasma from young (20−30 years old) and aged (60−90 years old) female donors. We found that supplementing the cells with aged plasma at the progenitor stage resulted in decreased cell expansion rate and subsequent differentiation capacity, along with reduced proliferation and increased apoptosis. Supplementation of aged plasma during terminal muscle differentiation led to formation of abnormally enlarged myotubes with mispositioned clusters of nuclei. Aged plasma did not affect cell commitment to myogenic lineage. We next examined our specific hypothesis that the aging effects came from age-specific changes in the levels of hypothalamus-pituitary-gonadal (HPG) hormones by conditioning the cells with bioidentical HPG hormones at the levels observed in pre-menopausal or post-menopausal women. Our preliminary data showed that imbalance of HPG hormones in post-menopausal women may be correlated to impairment in self-renewal and differentiation of myogenic progenitors. This study demonstrates the feasibility to simulate sarcopenia on a dish using hPSC-

derived myogenic progenitors and human plasma, thereby opening up possibilities to acquire new understanding about how changes in plasma components disrupt muscle homeostasis with age.

(12) Epigenetic control of transcription elongation at non-lineage specific genes enables induced pluripotency

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Pluripotent stem cells have a transcriptionally permissive epigenome that is less compacted, depleted for repressive modifications, and enriched for activating histone modifications as compared to somatic cells. A striking exception is DOT1L-mediated H3K79 methylation that is enriched on the gene bodies of highly transcribed genes, proportional to gene activity. Here we find that embryonic stem cells (ESCs) favor low H3K79me levels because it facilitates RNA polymerase II (RNAPII) elongation. Inhibiting the catalytic activity of DOT1L during the reprogramming of somatic cells to pluripotency confers both an ESC-like RNAPII profile and greater nascent transcription. DOT1L inhibition causes a local gain of histone acetylation at genes that lose the most H3K79me, which surprisingly are not lineage specifying genes, but ubiquitously expressed genes that perform essential functions in every cell. Maintenance of this elevated histone acetylation is required for the enhanced conversion of somatic to induced pluripotent stem cells. Together, we discover a novel epigenetic control mechanism promoting the hyperacetylation and hypertranscription hallmarks of pluripotency that governs transcription elongation at ubiquitously expressed genes to enforce cell fate.

GENERAL POSTER SESSION

(13) Exploring effects of hypoxia on blood-brain barrier property induction in naïve endothelium using hPSC-derived in vitro models.

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Induction of blood-brain barrier (BBB) properties in CNS endothelial cells during human development is incompletely understood and our knowledge of this process is derived mainly from animal models. In vitro BBB models can be used to model human BBB development and cerebrovascular disease. We use developmentally relevant cell types and signaling inputs, including a hypoxic microenvironment, to improve BBB models. We modeled development in vitro by differentiating human pluripotent stem cell (hPSC)-derived endothelial progenitors to naïve endothelial cells (hPSC-naïve ECs) in medium conditioned by hypoxic hPSC-derived neural progenitors, one component of the developmental “neurovascular unit”. We determined the effect of hypoxia on secretion of soluble factors from the neural progenitors and their impact on BBB property induction in hPSC-naïve ECs. Previous studies showed that Wnt signaling mediates neural progenitor-EC communication during BBB formation; Wnt activation increases GLUT-1 (BBB-enriched glucose transporter) expression and reduces PLVAP (transcytosis protein) expression. Wnt activation paradoxically increases expression of caveolin-1, another transcytosis-associated scaffolding protein. We show that hypoxia-treated neural progenitors may secrete a soluble factor that appropriately reduces caveolin-1 expression in hPSC-naïve ECs,

likely independent of Wnt signaling. These findings suggest that hypoxia could enhance secretion of soluble BBB-inducing factors from developmentally relevant CNS cell types. We propose methods to identify such a factor like qPCR, growth factor and cytokine arrays, and quantitative mass spectrometry. Novel signaling pathways for BBB property induction in CNS ECs can be deduced by transcriptomic and proteomic investigation of cells and conditioned medium.

(14) Identification of *Hox11* genomic binding sites in skeletal stem cells using novel epitope-tagged alleles

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Mammalian *Hox* genes are essential for the proper development of the axial and appendicular skeleton during embryogenesis. Posterior *Hox* genes (*Hox9-13*) are critical for patterning the skeletal elements of the limb along the proximodistal axis. *Hox11* paralogous genes pattern the zeugopod, which is comprised of the radius/ulna and the tibia/fibula in the forelimb and hindlimb, respectively. Recent studies have demonstrated that *Hox11* is exclusively expressed in skeletal stem cell population throughout life where they regulate the early differentiation to both chondrocytes and osteoblasts. Despite well understood genetic contributions to limb development, maintenance and repair, the mechanisms by which *Hox11* regulates skeletal stem cells remain unknown. Using our newly generated *Hoxa11-3XFLAG* and a *Hoxd11-3XFLAG* epitope-tagged knock-in alleles we aim to define the chromatin binding profile of *Hox11* in skeletal stem cells and in early chondrogenesis and osteogenesis. We have validated these alleles by PCR, sequencing, immunofluorescence and western blotting. $Hoxa11^{3XFLAG/3XFLAG}$; $Hoxd11^{3XFLAG/3XFLAG}$ animals demonstrate no overt patterning defects and these animals are fertile (unlike single mutant animals for either *Hoxa11* or *Hoxd11*). Additionally, we have confirmed *Hox11-3XFLAG* binding to a known *Hox11* downstream target, a *Six2* enhancer in the developing kidney, using CUT&RUN assays and ChIP qPCR. Our preliminary studies indicate that these new alleles are useful to perform DNA-protein binding analyses and will allow us to identify enhancers and potential direct downstream transcriptional targets of *Hox11* to understand their function during skeletal patterning.

(15) Chemically defined substrates in a ready-to-use format that promote differentiation, accelerated maturation and neurite extension of multiple neuronal subtypes for screening applications

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Human neural cells manufactured from induced pluripotent stem cells (iPSCs) hold great promise for modeling neurodevelopmental disorders, screening for potential risks from environmental toxins, and use in regenerative cell therapies. However, most protocols require the use of complex, animal-derived substrates that need to be prepared prior to cell plating and introduce variability to culture outcomes. Stem Pharm has developed chemically defined substrates employing norbornene-functionalized polyethylene glycol (PEG) and synthetic peptides that can be applied to cell culture surfaces, dehydrated, packaged, sterilized, and stored, ready for re-hydration and use. We employed Design of Experiment (DOE) methodology

utilizing Box-Behnken response surface modeling to screen for formulations that promoted cell viability, adhesion, and desired morphology. Our results indicate accelerated maturation of cortical glutamatergic and motor neurons as well as differentiation from neural precursor cells to glutamatergic neurons. These formulations were evaluated for compatibility with commercially available iPSC-derived neurons including GABAergic neurons and transcriptionally induced excitatory neurons. Transcriptional profiling using bulk RNASeq analysis was performed to compare neural cultures on the substrates with those cultured on standard substrates, including charged polymers (poly-lysines) or animal-derived substrates (PLO-Laminin or Geltrex). Cells cultured on Stem Pharm's hydrogel substrates demonstrated an increase in expression of gene ontology sets for neuronal maturation, neuron projection guidance, and cell-substrate adhesion in day seven samples: and enhanced cell differentiation, migration, signaling at the cell periphery and vesicles at day fourteen. The pre-plated substrates reduce time for experimental preparation, reduce the substrate complexity, eliminate ethical considerations of animal-derived substrates, and improve assay metrics when applied to screening campaigns.

(16) hPSC-derived Epicardial Cell Behavior in Coculture with hPSC-derived Cardiomyocytes

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Epicardial cells (EpiCs) are necessary for proper heart formation, however little is known about crosstalk between EpiCs and cardiomyocytes (CMs) during development and the potential impact of EpiCs on CM maturation. To investigate the effects of EpiCs on CM commitment and maturation, we differentiated human pluripotent stem cells (hPSCs) to cardiac progenitor cells (CPCs) and EpiCs, and cocultured EpiCs and CPCs for two weeks. We identified changes in CM structural maturation including sarcomere organization and induced proliferation, and we demonstrated that these changes are only observed in direct coculture and that conditioned medium or indirect coculture is insufficient. Finally, single cell sequencing of EpiC cocultures had bi-directional effects and biased EpiC lineages. This work suggests important crosstalk between EpiCs and CMs during differentiation can be used to influence cell fate and improve the ability to generate cardiac cells and tissues for *in vitro* models and development of cardiac cellular therapies.

(17) Brain and Organoid Manifold Alignment (BOMA), a machine learning framework for comparative gene expression data analysis across brains and organoids

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Brain organoids, differentiated from pluripotent stem cells, have become useful models for understanding gene expression and functions that govern brain development. Various mechanisms may affect the development of organoids, for example, cell competitions, etc. However, the gene expression dynamics of organoid development and how well they mimic the human brains development, especially at cell level, remains unclear. Importantly, there is a lack of dedicated computational approaches for comparative analyses between in vitro and in vivo

data. To address this, we have developed a machine learning framework, Brain and Organoid Manifold Alignment (BOMA), to align samples of brains and organoids and identify conserved/specific developmental trajectories as well as expressed genes and functions, especially at cellular resolution. BOMA first performs a global alignment and then uses manifold learning to locally refine the alignment. Such aligned samples reveal conserved developmental trajectories between brains and organoids (or specific trajectories from unaligned samples). We applied BOMA to align various bulk-tissue and single-cell RNA-seq datasets to show its generality and scalability. We found that human cortical organoids better align with certain brain cortical regions than other and non-cortical regions, implying organoid-preserved developmental gene expression programs specific to brain regions. Additionally, our alignment on non-human primate versus human organoids revealed species discrepancies during their development. Also, we integrated developmental scRNA-seq data of human brains and organoids and showed conserved/specific cell trajectories and clusters. The enrichment analyses of differentially expressed genes of those clusters reveal brain/organoid-specific developmental functions and pathways. In summary, BOMA comparative analyses not only uncovered the discrepancies between brain and organoid development, but also constructed cell level developmental trajectory for organoids. These trajectories will benefit the understanding the mechanisms during organoid development, including cell competition, etc. BOMA is available as open-source and web tools, which allows community users to align their own organoid RNA-seq datasets.

(18) Pathogenic Mutation that Dislocates GATA2 Zinc Fingers Establishes a Hematopoiesis-Disrupting Signaling Network

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While certain human genetic variants are conspicuously loss-of-function, decoding the functional impact of many variants is challenging. Previously, we described a leukemia predisposition syndrome (GATA2-deficiency) patient with a germline GATA2 variant that inserts nine amino acids between the two zinc fingers (9aa-Ins). Here, we conducted mechanistic analyses using genomic technologies in Gata2 -77 enhancer-mutant hematopoietic progenitor cells to reveal how the insertion impacts GATA2 function genome-wide. Despite being nuclear-localized, 9aa-Ins was severely defective, with activation more impaired than repression. Variation of the inter-zinc finger spacer length revealed that repression tolerated insertions that were detrimental to activation. GATA2 deficiency generated a hematopoiesis-disrupting signaling network in progenitor cells with functional alterations in multiple cytokine signaling systems, including those predicted to cause hallmark phenotypes of GATA2 deficiency

syndrome. Mechanistic analyses conducted with additional GATA2 disease-linked variants confirmed and extended these conclusions. These results establish principles underlying GATA factor function in physiological and pathological contexts, and comparable systems are being deployed to elucidate the mechanistic basis of blood cancer predispositions involving genetic variation in other hematopoietic-regulatory genes.

(19) Potential therapies for Kir7.1 channelopathy

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Purpose

Leber Congenital Amaurosis 16 (LCA16) is a monogenic inherited ocular channelopathy caused by several biallelic point mutations in KCNJ13, which affect the retinal pigmented epithelial (RPE) layer of the retina. W53X (c.158G>A; tGg>tAg) mutation in KCNJ13 results in a truncated Kir7.1 protein. In the current study we aim to use two therapeutic approaches to restore the Kir7.1 channel function in W53X-LCA16-patient derived iPSC RPE. 1). clinical grade AAV5-Kir7.1 HUB101 gene-therapy. 2). Adenosine-CRISPR-base editor (ABE), delivered by silica nanoparticles (SNP). The outcomes of these two therapies were compared alongside for potency, efficiency, and safety.

Methods

1. W53X iPSC RPE cells were transduced with three doses (2, 5, and 10 ul) of AAV5-Kir7.1(0.5x1013gc/ml) to study the dose-dependent expression of transgene. Kir7.1 and immune markers expression (by qPCR), protein expression and localization (by immuno-assays), and function (by manual and automated patch-clamp; APC). Untreated and isogenic cells were used as control.

2. Efficiency of ABEs (mRNA and RNP) was tested in HEK293 stable cells expressing W53X-Kir7.1 and LCA16 patient's derived fibroblasts. To edit W53X iPSC RPE cells, ABE mRNA (ABE8e-spCas9-NG) along with a modified sgRNA (3:1 molar ratio) targeting W53X mutation was delivered using SNPs. Untreated cells were used as reference. On-target/off-target editing efficiency was evaluated by deep sequencing using Illumina-next generation sequencing platform. electrophysiology was done to evaluate the Kir7.1 channel function in base-edited iPSC RPE.

Results

1. Expression profile of AAV5-Kir7.1 transduced iPSC RPE cells indicated dose-dependent

expression of Kir7.1 transcript. Protein expression confirmed the successful translation of exogenous Kir7.1 and trafficking to the membrane. Electrophysiology assay showed the rescue of Kir7.1 protein function in transduced cells, comparable to isogenic iPSC RPE cells.

2. The screening of ABEs (mRNA and protein as RNP) for W53X (TaG>TgG) correction in HEK293 stable cells showed that the mRNA approach (50% efficiency) is better than RNP (25%). Nanoparticle-mediated delivery of ABE-mRNA in fibroblasts (47%) and post-mitotic hiPS-RPE (20%) established efficient therapeutic base editing that could now be undertaken for in vivo BE experiment. On target Indel mutagenesis (<3%) and deep sequencing of potential off-target sites (<1%) reassured the safety of ABEs. Electrophysiology showed the rescue of channel function in the edited iPSC RPE.

Conclusions

- The expression, safety, toxicity, and functional studies in AAV5-Kir7.1 treated cells confirmed the clinical use of AAV5 serotype to transduce outer retinal layer.
- ABE delivered by SNPs showed the potential to precisely correct the point mutations with reduced or no off-targets, and overcome the adverse effect of AAV-associated immune response.
- Functional restoration in transduced and edited cells suggests the potential of this therapies in treatment of pediatric blindness with significant outcomes.
- Although, endogenous gene correction via ABE has the upper hand (long-term and permanent change) over exogenous supplementation of Kir7.1 via AAVs, it requires mutation-specific design to correct disease phenotype. However, AAV-gene augmentation is a universal approach and beneficial for hard to edit mutations because of sequence complexity.
- Channelopathies like LCA16 require bi-allelic gene correction in the diseased cell to produce a multimeric functional channel.

(20) A Scalable Human Neural Rosette Assay™ for Assessment of Environmental and Genetic Neural Tube Defect Risk

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Neural tube defects (NTDs) are the second most common congenital malformation in the US and beyond. Given the multifactorial etiology and complicated genetic landscape underlying NTDs, current *in vivo* and *in vitro* models of neural development have limited utility for investigating clinical NTD pathophysiology and discovering preventative interventions. This is further confounded by species differences and lack of scalability with typical animal models. Human pluripotent stem cell (hPSC)-derived neural organoids partially address these shortcomings as they recapitulate incipient neural morphogenesis, i.e., neural tube formation, in vitro. However, their spontaneous emergence causes inconsistencies in tissue composition and cytoarchitecture, thus limiting their application. We hypothesized that this variability is a of product of non-standard and insufficiently controlled biophysical and biochemical cues. As such,

we developed a microarray culture platform that enables spatiotemporal control of microenvironmental cues to reproducibly direct early neural organoid morphogenesis. The resulting human Rosette Array™ platform standardizes derivation of 3-D, singularly polarized neuroepithelial rosette tissues from various rostral/caudal CNS regions with high efficiency and reproducibility. Here, validation of the Rosette Array platform for environmental and genetic NTD risk assessment is presented. Using neural rosette emergence as a principal metric, various pharmaceuticals and agrochemicals known to cause NTDs through multiple modes of action were screened to evaluate the Rosette Array's ability to assess NTD risk; results displayed high sensitivity and specificity in line with similar *in vitro* assays. This includes a demonstrated ability to detect disruption of the developmentally critical folic acid metabolism pathway. Additionally, preliminary experiments using gene edited hPSCs containing clinically-relevant NTD mutations showed that the platform could detect correlated perturbations to neural rosette morphogenesis in a CNS region-specific manner. Thus, the Rosette Array platform is a scalable, "off-the-shelf" screen for quantitatively assessing clinical NTD risk and prophylactics. This technology has direct regulatory applications, and future work involving NTD patient-derived hPSCs evoke the possibility of patient-specific risk assessment and discovery of personalized prophylactics.

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Conflict of interest disclosure: RSA, GTK, and RMW are co-founders of Neurosetta LLC, which seeks to commercialize the presented technology.

(21) Voltage-gated sodium channel, scn8a, is required for innervation and regeneration of amputated adult zebrafish fins

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Teleost fishes and urodele amphibians can regenerate amputated appendages, whereas this ability is restricted to digit tips in adult mammals. One key component of appendage regeneration is reinnervation of the wound area. However, how innervation is regulated in injured appendages of adult vertebrates remains largely unknown. From a forward genetics screen for temperature-sensitive defects in zebrafish fin regeneration, we identified a mutation that disrupted regeneration while also inducing paralysis at the restrictive temperature. Genetic mapping and complementation tests identified a mutation in the major neuronal voltage-gated

sodium channel (VGSC) α-subunit gene *scn8ab*. Conditional disruption of *scn8ab* impaired early regenerative events, including blastema formation, but did not affect morphogenesis of established regenerates. Whereas *scn8ab* mutation leads to a reduction of neural activity as expected, disrupted axon growth and patterning in fin regenerates was also observed, resulting in hypo-innervation. Our findings indicate that the activity of voltage-gated sodium channels plays pro-regenerative roles by promoting innervation of appendage stumps.

(22) Lactate-based Purification of Human iPSC-Derived Cardiomyocytes Does Not Alter Sarcomeric Proteome or Function of Engineered Cardiac Tissue

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Human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) are commonly utilized in vitro models for heart disease. Three-dimensional engineered cardiac tissue (ECT) constructs made from hiPSC-CMs have emerged as appealing model systems due to their closer representation of complexity of the heart. Recent studies have suggested that metabolic (lactate) purification of 2-dimensional hiPSC-CMs result in an ischemic phenotype with aberrant contractile parameters and altered sarcomere phosphorylation1. Herein, our objective was to investigate if these phenotypes hold true in lactate purified ECTs. hiPSCs were differentiated into hiPSC-CMs and then purified using either a lactate-based media or magnetic antibody-based purification depleting the nonmyocyte cell population. Both purification approaches generated hiPSC-CM cultures with greater than 98% purity. After purification, hiPSC-CMs were combined with hiPSC-cardiac fibroblasts to create 3D ECT constructs (n=6 for lactate, n=3 for magnetic) and maintained in culture for four weeks. Isometric twitch force measurements were performed to characterize contractile performance between the lactate and magnetic purification ECTs. Using the exact same ECT, sarcomere proteins were extracted and analyzed using a top-down liquid chromatography-mass spectrometry-based approach to quantify sarcomere protein isoforms. Functionally, the lactate ECTs displayed similar magnitude of twitch force and similar overall contraction and relaxation times.Interestingly, the lactate ECTs displayed a slightly shorter time to 50% contraction over the magnetically purified ECTs. Sarcomeric proteoforms from the functionally tested ECTs were found in the same percentage of total phosphorylation for contractile proteins α-tropomyosin, myosin light chain 2v, and cardiac troponin T between conditions. Quantitation of these proteins also revealed no significant changes in sarcomeric protein levels. Our results reveal that lactate selection of hiPSC-CMs generate 3D ECTs constructs with phenotypes similar to magnetically selected hiPSC-CMs and suggest that lactate hiPSC-CMs may recover in three-dimensional constructs with time in culture.

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Predicting Ligands That Direct T cell Maturation

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There is great interest in using induced pluripotent stem cell (iPSC) derived T cells for off-the-shelf immunotherapy[1]. Methods for differentiating T cells from pluripotent stem cells rely primarily on undefined mouse cocultures that leave cells mostly arrested at an immature stage *in vitro*[2]. The unique cues that are important for T cell development *in vitro* are largely unexplored beyond the reliance on Notch signaling. Notch ligands are typically presented by surrounding stromal cells as delta-like ligands 1 and 4 (DLL1 and DLL4), forming the basis for *in vitro* differentiation methods such as the OP9-DLL4 coculture[2]. Quantitative analysis of T cell development in the thymus could shed light on what signals are presented to thymocytes during development. Comparing thymic signals to those in *in vitro* differentiation systems can help to inform the engineering of the *in vitro* differentiation niche to generate more mature iPSC derived T cells.

Gene expression of the postnatal human thymus[3,4] and the top differentially expressed genes between naïve and *in vitro* derived CD8 T cells[5,6] were used to model cell-cell communication[7]. Ligands that impact the differences in gene expression between *in vitro* derived and naïve T cells were predicted. The regulatory potential of prioritized ligands over pseudotime[8] was plotted to achieve a temporal prediction of cell-cell signaling the thymus (Fig 1). Based on this analysis, we predict that knocking out ligands such as BMP4 and BMP7 in later stages of directed differentiation, while adding IL6, IL7, and CXCL12 in the later stages may improve maturation of T cells *in vitro.* Interestingly, Notch ligands are not in the top hits, perhaps due to the wide presence of these ligands *in vitro*. The next steps are to perform a screen to knock out or overexpress these ligands in the OP9-DLL4 platform to improve T cell maturation.

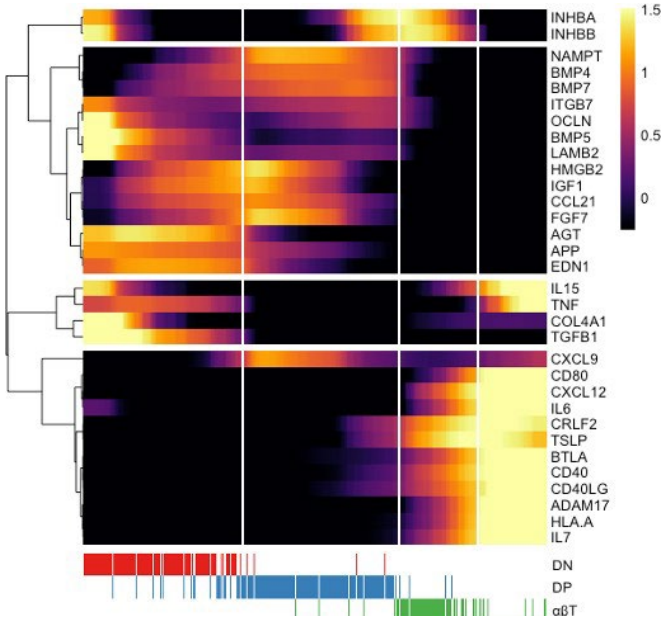

Figure 1. Regulatory potential of top ligands during human T cell development. Using cell-cell communication and pseudotemporal modeling, the regulatory potential of ligands that influence the differences in *in vitro* and *in vivo* derived T cells are shown along the differentiation trajectory (bottom, starting from double negative (DN) cells to CD8+ T cells). Higher regulatory potential (brighter in top right color scale) means a stronger regulation of the target genes.

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(24) Validating phenotypic beta cell maturation markers in human pancreas development

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Stem cell-derived islets (SC-islets) hold promise as a beta cell replacement therapy to treat diabetes. After transplantation, SC-islets attain improved maturation and functional profiles compared to *in vitro* culture. The acquisition of maturation is important in these cells in order to properly secrete insulin to control blood glucose levels; under-secretion of insulin will not be sufficient to reverse diabetes, while over-secretion of insulin could cause deadly hypoglycemia. One method for measuring maturation in SC-islets is based on function, but functional assays are time consuming, expensive, and require high numbers of living cells. The identification of markers for human beta cell functional maturation would therefore be informative for improving SC-islet differentiation, could facilitate the sorting of more mature beta cells from the pool of differentiated cells, and will stimulate a better understanding of normal human islet development. While several candidate factors to mark beta cell maturation have been identified, much of the data supporting these markers come from animal models or SC-islets; it is unknown how well these expression profiles mirror primary human islet development. One such marker is Urocortin-3 (Ucn3), which is expressed only postnatally in mice when islets become functional. We explored Ucn3 expression at the gene and protein level in human fetal pancreas (HFP), adult human islets (AHI), and SC-islets and found that Ucn3 is expressed in human fetal islets well before the acquisition of functional maturation, and show that SC-islets that express Ucn3 (Protocol B) are no more functionally mature than those that have no Ucn3 expression (Protocol A) (Figure 1). We then utilized our tissue bank and SC-islet resources to test an array of other candidate maturation markers, and identified NTPDase3, G6PC2, IAPP, MAFA, SIX2, and SIX3 as markers with expression patterns that correlate developmentally with the onset of functional maturation in human islets.