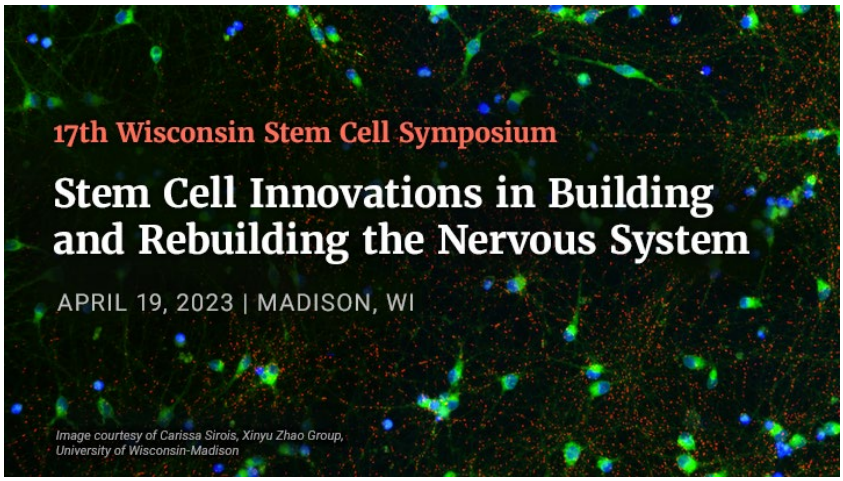

NOTE: Each submitter's name is in bold and italicized. Travel awardees are indicated in red.

POSTER CONTEST FINALISTS

1 Directed differentiation of human pluripotent stem cells into neurovascular unit cell types via transcription factors

Yunfeng Ding[1], Soniya Tamhankar[1], Tessa Christopherson[1], Jenna R. Cohen[1], Nate Schlueter[1], Sean P. Palecek[1] and Eric V. Shusta[1,2]

[1]Department of Chemical and Biological Engineering, University of Wisconsin – Madison, Madison, WI, USA
[2]Department of Neurological Surgery, University of Wisconsin – Madison, Madison, WI, USA
ding52@wisc.edu

The blood-brain barrier (BBB) is a selective semipermeable membrane that separates the central nervous system (CNS) from the bloodstream, tightly regulating the exchange of substances between these two compartments. Brain microvascular endothelial cells (BMECs) are a key component of the BBB and work in concert with pericytes, astrocytes, neurons, and microglia to form the neurovascular unit (NVU), which helps maintain the BBB's integrity and function. To investigate the potential of human pluripotent stem cells (hPSCs) as a source of BMECs, we developed methods to differentiate hPSCs into induced endothelial cells (iECs), neurons (iNs), or neural progenitor cells (iNPCs) by inducible overexpression of transcription factors ETV2, ATOH1, or ASCL1. We used flow cytometry and immunocytochemistry to characterize the differentiated cells based on the expression of canonical cell type markers. We then explored whether co-differentiation of iECs with iNs or iNPCs could promote BMEC-like phenotypes, which would be a crucial step in developing a functional NVU model. Furthermore, we optimized the differentiation of iECs by varying seeding densities, soluble factors, and the length of ETV2 induction. Our efforts led to the development of a differentiation scheme that produced nearly pure iECs (>98% CD31+) in just five days, without the need for additional purification steps. Additionally, we identified soluble factor combinations that induce expression of a subset of BMEC-related genes in iECs. In future studies, we will further refine our differentiation strategies to enhance BMEC-related phenotypes and establish an *in vitro* NVU model based on ETV2-iECs and other induced NVU cell types.

2 Age-specific imprinting through direct reprogramming reveals a developmental loss of intrinsic neurite growth ability in human neurons

Bo P. Lear*[*1], Elizabeth A.N. Thompson*[*1], Kendra Rodriguez[1], Zachary P. Arndt[1], Saniya Khullar[1,2], Payton C. Klosa[1], Ryan Lu[3], Christopher S. Morrow[1], Bryan Teefy[3], Daifeng Wang[1,2,] Ryan R. Risgaard[1], Andre M.M. Sousa[1], Berenice Benayoun[3], Darcie L. Moore[1]

* Indicates equal contributions

1 Department of Neuroscience, University of Wisconsin-Madison, Madison, WI

2 Department of Biostatics and Medical Informatics, Department of Computer Science, University of Wisconsin-Madison, Madison, WI

3 Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA

bpeng26@wisc.edu

Spinal cord injury (SCI) leads to life-long disability, with limited treatment options. After injury, central nervous system (CNS) axons fail to regenerate due both to extrinsic and intrinsic factors. Rodent studies have revealed a developmental regulation of axon growth ability, such that embryonic CNS neurons extend long axons, whereas postnatal CNS neurons cannot. Yet, whether this is similar in human CNS neurons is unknown. Recently, our lab generated an *in vitro*, age-relevant, human model to identify novel intrinsic factors which regulate axon growth. This direct reprogramming protocol transdifferentiates human fibroblasts directly into neurons (Fib-iNs), skipping pluripotency which restores cells to an embryonic state. Using human fibroblast samples from 8 gestational weeks to 72 years-old, we confirmed that Fib-iNs maintained the original cell's age. Further, we found that early fetal Fib-iNs grew longer neurites relative to late fetal and adult ages, mirroring the age-dependent decrease in regenerative ability during development in rodents. Interestingly, these neurons are environmentally naïve, suggesting an intrinsic aging clock may drive changes in neurite growth ability. Using RNA-sequencing on all ages of Fib-iNs, we identified dramatic transcriptional shifts between ages with high versus low intrinsic growth ability. We performed a small screen to identify regulators of neurite growth based on these differentially expressed genes, and identified ARID1A, a subunit of the BAF nucleosome remodeling complex, to be a key developmentally regulated gene that drives neurite outgrowth. These results suggest that age-specific imprinting at the chromatin level may be the driver for the intrinsic loss of neurite growth ability in human CNS neurons during development. Taken together, we characterized neurite outgrowth in human neurons, providing a new resource for future studies in axon growth and regeneration that can be used to identify novel therapeutic candidates for SCI.

3 Modeling Clinically Relevant Neural Tube Defect Risk using RosetteArray™ TechnologyPresenting

Brady F Lundin[1], Gavin T Knight[1,2,3], Nikolai Fedorchak[2,3], Joshua F. Robinson[4], Bermans J. Iskandar[5], Rebecca M Willet[3,6], and Randolph S Ashton[1,2,3]

[1] Department of Biomedical Engineering, University of Wisconsin-Madison

[2] Wisconsin Institute for Discovery, University of Wisconsin-Madison

[3] Neurosetta LLC, Madison, Wisconsin

[4] Department of Obstetrics, Gynecology & Reproductive Sciences, University of California-San Francisco

[5] Department of Neurological Surgery, University of Wisconsin-Madison

[6] Department of Computer Science, University of Chicago

blundin@wisc.edu

Neural tube defects (NTDs) are the second most common congenital malformation with a complicated multifactorial etiology, and standard in vitro models using mainly gene-altered rodents have limited scale and non-human background. Current rodent models do not accurately depict the human presentation of NTDs, with spinal defects dominating in human patients, while the vast majority of rodent models display embryonic lethal failure in forebrain

neural tube closure. Additionally, no single gene has been identified that reproducibly causes isolated NTDs[1], yet multiple genomic mutations contribute to these defects making it challenging to estimate susceptibility based on individual variants with existing models. To address these limitations, we developed a standardized and quantifiable model of neural tube closure using human pluripotent stem cell (hPSC) derived RosetteArrays that enable modeling of discrete central nervous system regional identities, i.e. forebrain vs spinal cord. By screening CRISPR edited hPSCs containing clinically associated genetic mutations within either the folate metabolism or planar cell polarity pathways, we have demonstrated quantifiable NTD-like phenotypes. Our study presents the platform's ability to model and quantify a clinically relevant multifactorial NTD scenario where the folate pathway mutated hPSC line demonstrates an increased sensitivity to folate pathway inhibitor Methotrexate compared to its isogenic control. Additionally, we have shown the planar cell polarity pathway mutated hPSC line's spinal region-dependent phenotype, highlighting the importance of regional identity in modeling human NTD risk. Our preliminary results demonstrate the RosetteArray's ability to model and quantify NTD susceptibility based on individual genetic variants in a human etiological background. Future work involving spina bifida patient-derived iPSCs evokes the possibility of personalized risk assessment and the development of precision medicine treatment approaches.

COI: RSA, GTK, and RMW are co-founders of Neurosetta LLC, which is commercializing RosetteArray technology.

References: 1. Iskandar, B. J., Finnell R.H. *N Engl J Med*. 387(5): 444-450 (2022). 2. Knight, G. T. *et al. eLife* 7, e37549 (2018).

4 A Trisomy 21 Human Stem Cell Model of Basal Forebrain Cholinergic Neurons

Jose Martinez1,2, Andrew J. Petersen2, Ke Xu2, Zhong-Wei Du2, Su-Chun Zhang2,3,4, Anita Bhattacharyya2,5

1. Cellular and Molecular Biology Graduate Program, University of Wisconsin, Madison, Wisconsin

2. Waisman Center, University of Wisconsin, Madison, Wisconsin
3. Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin
4. Department of Neurology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin
5. Department of Cell and Regenerative Biology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin

jlmartinez4@wisc.edu

Down syndrome (DS, Ts21), is characterized by intellectual impairment at birth and Alzheimer's disease (AD) pathology in age. Basal forebrain cholinergic neurons (BFCNs) are a neuronal population critical for memory and cognition. They're vulnerable to degeneration in DS and AD. Key molecular pathways involved in BFCN development and degeneration are poorly understood. In vitro generation of BFCNs from hPSCs is a valuable method by which to investigate and identify these factors. Few protocols to derive BFCNs from hPSCs have been established. Thus, we used a hESC line, H9, to develop a protocol that mimics key transcription factor expression in BFCN neural progenitor cells. BFCNs develop from LHX8 and ISLET1-expressing progenitors in the ventral telencephalon, and ultimately express choline acetyl transferase (ChAT). We first modified existing protocols with addition of SHH and validated that early addition of SHH results in robust NKX2.1 expression, indicative of ventralization. BFCN progenitor fate specification (LHX8 expression) was manipulated by addition of NGF at different timepoints. Results showed a time dependent increase in expression of LHX8.We also found that addition of BMP9 results in an increase in ISLET1 expression, indicating a progression toward

mature BFCNs. Together these results provide a new strategy to differentiate hPSCs to BFCNs. To determine whether there are differences in the generation of BFCNs in isogenic pair of Ts21

and control iPSCs, we built ChAT-P2A-Cre mCherry reporter lines. We then employed our newly developed protocol to generate BFCNs, and we found that Ts21 iPSCs generated less NeuN+ neurons as compared to isogenic controls. These results suggest that BFCNs in DS may have altered development that results in fewer BFCNs, which in turn may affect the impact of their neurodegeneration as fewer BFCNs will be spared. We provide a new model to explore differences of BFCNs derived from control and disease specific hPSCs.

5 PREDICTING AND ENHANCING CARDIAC POTENTIAL OF IPSC-DERIVED CARDIAC PROGENITOR CELLS

***Aaron D. Simmons1*,** Austin Feeney2, Claudia Baumann3, Xiangyu Zhang3, Rabindranath De La Fuente3, Sean P. Palecek1,2

1University of Wisconsin Department of Chemical and Biological Engineering
2University of Wisconsin Department of Biomedical Engineering
3University of Georgia Department of Physiology and Pharmacology

adsimmons2@wisc.edu

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) exhibit marked potential for use in cell therapies, drug screening, and disease modeling applications. The efficiency of their production; however, remains quite variable resulting in poor process robustness. Thus, methods to monitor this differentiation, predict final CM yield and purity at earlier stages, and enhance these metrics will significantly increase process reliability and productivity. Cardiac progenitor cells (CPCs) are a key metastable intermediate in this process and thus present an opportune cell type to study. Herein, we have undertaken a multi-omics discovery approach to identify early differences in cell attributes between high- and low-potency CPCs batches (i.e. those giving rise to high- and low-purity populations of hPSC-CMs) to provide systems-level insights into underlying mechanisms which drive these divergent outcomes. Specifically, we have combined epigenomic (ATACseq), transcriptomic (RNAseq), proteomic, and lipidomic profiling methodologies. To date, we have identified differential molecular and functional signatures between high- and low- potency CPCs with high power to predict terminal hPSC-CM purities. Among these signatures, in addition to confirming some canonical cardiac developmental genes (NKX2-5, CRIP2) being enriched in high-potency CPCs, we have also identified marked differences in WNT, MAPK, and EMT pathways, epigenetic priming of key sarcomeric-related genes, lipid storage, and oxidative metabolism (Seahorse assay). Furthermore, through inferential dynamics we have identified cell-intrinsic speed of differentiation to be a potential significant contributor to divergent terminal hPSC-CM outcomes. Finally, we are beginning to utilize these data to enhance hPSC-CM differentiation outcomes (yield and purity). Thus far, in addition to gaining fundamental insights into the underlying biology of CPCs, our findings are being used to 1) predict final hPSC-CM differentiation process outcomes early, 2) enhance these process outcomes through process optimization, and 3) establish potential novel feedback control schemes to further enhance process robustness.

6 CGG repeats in the *FMR1* gene regulate mRNA and cellular stress in developing neurons

***Carissa L. Sirois*[1,2]**, Yu Guo,[1,2], Meng Li,[1,2], Tomer Korabelnikov[1,2], Soraya Sandoval,[1,2] Amaya Contractor,[1,2,] Natalie Wolkoff,[1,2] Minjie Shen,[1,2] Yina Xing,[1,2] Bradley Levesque,[1,3] Andre M.M. Sousa,[1,2] Anita Bhattacharyya,[1,3] Xinyu Zhao[1,2]

[1] Waisman Center, University of Wisconsin-Madison, Madison, WI

[2] Department of Neuroscience, University of Wisconsin-Madison, Madison, WI

[3] Department of Cell & Regenerative Biology, University of Wisconsin-Madison, Madison, WI

clsirois@wisc.edu

FMR1 (Fragile X messenger ribonucleoprotein 1) is a gene important for neuronal development and function, the misregulation of which can lead to several disorders, including Fragile X Syndrome (FXS). The 5' UTR of *FMR1* contains polymorphic CGG repeats, the length of which can have differing effects on *FMR1* gene expression and FMRP protein levels. While repeats below a certain threshold were typically assumed to not have any major effect on *FMR1* expression, recent works have found associations between "normal" repeats and several aspects of human health. However, the function of normal CGG repeats in brain development remains unknown. Here, we have examined the function of normal length CGG repeats in the *FMR1* 5' UTR by comparing neurons with normal repeats (31 CGG) to isogenic control neurons in which the CGG repeats have been removed (0 CGG). We found that removal of these repeats leads to altered levels of cellular stress proteins and differential response of neurons to glucocorticoid receptor (GR) activation. Removal of CGG repeats also leads to increased export of *FMR1* mRNA to dendrites and mislocalization of several FMRP target mRNAs, both at baseline and in response to GR activation. These results indicate that CGG repeats play an important role in several aspects of early neuronal development, and indicate a potential mechanism by which different length CGG repeats in the "normal" range lead to differences in human patients.

7 Generation of Locus Coeruleus Norepinephrine Neurons from Human Pluripotent Stem Cells

***Yunlong Tao*[1],** Xueyan Li[1], Qiping Dong[1], Linghai Kong[1], Andrew J. Petersen[1], Yuanwei Yan[1], Ke Xu[1], Seth Zima[1], Yanru Li[1], Danielle K. Schmidt[1], Melvin Ayala[1], Sakthikumar Mathivanan[1], Andre M. M. Sousa[1,2], Qiang Chang[1,2] and Su-Chun Zhang[1,2,3]

1Waisman Center, University of Wisconsin-Madison, Madison, WI, USA

2Department of Neuroscience, Department of Neurology, University of Wisconsin, Madison, WI, USA

3Program in Neuroscience and Behavioral Disorders, Duke-NUS Medical School, Singapore

taoyunlong@gmail.com

Central norepinephrine (NE) neurons, mainly located in the Locus coeruleus (LC), play roles in a wide range of behavioral and physiological processes. How the human LC-NE neurons develop and what roles they play in the pathophysiology of human diseases is poorly understood, partly due to the unavailability of functional human LC-NE neurons. Here we established a technology for efficient generation of LC-NE neurons from human pluripotent stem cells by identifying a novel role of ACTIVIN A in regulating the LC-NE transcription factors in the dorsal rhombomere 1 (r1) progenitors. The in vitro generated human LC-NE neurons not only display extensive axonal arborization and release/uptake NE, but also exhibit the pacemaker activity, calcium oscillation, and in particular chemoreceptor activity in response to CO2. The LC-NE neurons engineered with a NE sensor reliably reported the extracellular NE level. The availability of functional human LC-NE neurons enables investigation of their roles in the pathogenesis of and development of therapeutics for neural psychiatric and degenerative diseases.

8 Investigating the role of fractalkine dysfunction in Alzheimer's disease pathology using a human induced pluripotent stem cell model

Kaylee D. Tutrow1,2, Melody Hernandez1,2, Katrina Whatley1,2, Jade Harkin2,3, Kang-Chieh Huang2,4, Jason S. Meyer1-3,5

1Department of Medical and Molecular Genetics, Indiana University School of Medicine
2Stark Neurosciences Research Institute, Indiana University School of Medicine
3Department of Pharmacology and Toxicology, Indiana University School of Medicine
4Department of Biology, Indiana University Purdue University Indianapolis
5Department of Ophthalmology, Indiana University School of Medicine

ktutrow@iu.edu

Dysfunctional microglial activity has recently been identified as a potential mechanism leading to accumulation of amyloid beta and Tau and subsequent neurodegeneration in Alzheimer's Disease. The CX3CR1/fractalkine axis serves as a mechanism for bi-directional communication between microglia and neurons, respectively, to promote a resting, anti-inflammatory state in microglia. Previous studies have demonstrated that deficiency in CX3CR1 signaling leads to a more pro-inflammatory phenotype in microglia, increased microglial reactivity and phagocytosis, and susceptibility to neuron death. Additionally, the CX3CR1 V249I variant was recently identified as a potential risk allele for Alzheimer's Disease with worsened Braak staging in post-mortem Alzheimer's patients. However, the mechanisms by which microglia with the CX3CR1-V249I SNP contribute to neurodegeneration remain unclear. Thus, to address this shortcoming, we utilized human induced pluripotent stem cells and CRISPR/Cas9 technology to elucidate the effects of the V249I variant on microglia-like cells compared to an isogenic control cell line. We demonstrate that microglia can be effectively differentiated from both isogenic control and CX3CR1-V249I backgrounds, and that microglia bearing the V249I allele demonstrated decreased phagocytosis of amyloid beta in vitro. These findings suggest that the CX3CR1 V249I variant may be associated with a pro-inflammatory, reactive phenotype in microglia which may contribute to neuronal dysfunction and death. Future work will expand upon these results to better delineate how this gene variant contributes to neurodegeneration related to Alzheimer's Disease.

9 Stem cell-derived astrocytes demonstrate perisynaptic astrocyte process defects and decreased synaptic-associated cell surface protein abundance in spinal muscular atrophy

Emily Welby1, Linda Berg Luecke2,3, Melinda Wojtkiewicz2, Rebekah L. Gundry2, Allison D. Ebert1

1Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA; 2CardiOmics Program, Center for Heart and Vascular Research; Division of Cardiovascular Medicine; and Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE, USA; 3Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, USA.

ewelby@mcw.edu

Spinal muscular atrophy (SMA) is characterized by the loss of the lower spinal motor neurons due to survival motor neuron (SMN) protein deficiency. The motor neuron cell autonomous and non-cell autonomous disease mechanisms driving early glutamatergic dysfunction, a therapeutically targetable phenotype prior to motor neuron cell loss, remain unclear. We recently demonstrated astrocyte-mediated diminishing of motor neuron activity by applying a microelectrode array approach to our induced pluripotent stem cell-derived co-culture system, which was likely caused by the decreased protein levels of the cell surface glutamate transporter, EAAT1, in SMA astrocytes. Microscale cell surface capture mass spectrometry experiments revealed a significant decrease in abundance of additional astrocyte plasma

membrane proteins associated with cell adhesion, synapse organization and regulation of neuron projection development in SMA astrocytes. Teneurin-4 (TENM4), recently demonstrated to be important in regulating astrocyte fine process morphology and outgrowth into the neuropil, was one of the most significantly low abundant cell surface proteins in SMA astrocytes. This is consistent with our recently characterized phenotype of fewer and disorganized perisynaptic astrocyte processes with reduced levels of neuromodulatory proteins in SMA cultures. We anticipate these defects in perisynaptic astrocyte processes likely mediate the astrocytic contribution to motor neuron dysfunction in SMA. Interestingly, while TENM4 transcript levels show no significant difference between healthy and SMA astrocytes, TENM4 protein levels were undetectable at the cell surface in 67% of SMA astrocyte technical replicates. Future studies will combine experimental approaches with computational modeling to assess if TENM4 mRNA transportation and local translation in perisynaptic astrocyte processes are defective in SMA astrocytes via an SMN-dependent mechanism.

10 Familial Alzheimer's Disease Patient iPSC-Derived Endothelial Cells Exhibit Dysregulated Angiogenesis and Altered Function

***Yu-Hsin Yen*[1]**, Cheong Meng Chong[2], Gabriel Chew[3], Hannah Wee[4], Christine Cheung[4], Su-Chun Zhang[1,5]

[1]Program in Neuroscience & Behavioral Disorders, Duke-NUS Medical School, 169857 Singapore, Singapore.

[2]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China.

[3]Programme in Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, 8 College Road, 169857, Singapore, Singapore

[4]Lee Kong Chian School of Medicine, Nanyang Technology University, Novena Campus, 11 Mandalay Road, 308232, Singapore

[5]Department of Neuroscience, Department of Neurology, Waisman Center, University of Wisconsin-Madison, Madison, WI 53705, USA.

E0316339@u.duke.nus.edu

Alzheimer's disease (AD) is a neurodegenerative disease characterized by an insidious onset of neurocognitive decline and hallmark histopathology of extracellular beta-amyloid (Aβ) plaques and intracellular tau tangles. Recently, a number of studies highlighted the presence of microvascular alterations and transcriptomic changes in endothelial cells (ECs) in both post-mortem AD patient brains and AD transgenic mouse models. In the AD mouse model, microvascular alterations can even be observed at postnatal day seven, suggesting early microvascular involvement in AD. However, how exactly do ECs contribute to AD in humans and whether these changes are a result of AD or a factor that contributes to AD development remain to be elucidated. We hypothesize that dysregulated EC function results in microvasculature deficits in the brain, contributing to AD pathogenesis. Deriving ECs from induced pluripotent stem cells (iPSCs) of familial Alzheimer's Disease patients with Presenilin-1 mutations and their isogenic pairs, we showed that ECs display increased proliferation, migration, and tube formation capabilities and reduced barrier integrity. Furthermore, our bulk RNA-seq data showed that PS1 FAD-iPSC ECs are transcriptionally more similar to those of AD patients than healthy controls. Interestingly, some altered genes in PS1 FAD-iPSC ECs are related to astrocyte-EC interactions, prompting further investigation of the involvement of blood-brain barrier in AD. Together, our results suggest that PS1 FAD-iPSC ECs are innately altered and may contribute to vasculature deficits seen in AD via pathological angiogenesis.

GENERAL SESSION

11 IL-1ra and CCL5 as targets for treating SMA astrocyte-mediated pathology

***R. L. Allison*[1],** A. D. Ebert[1]

1. Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI

rallison@mcw.edu

Spinal muscular atrophy (SMA) is a leading genetic cause of infant mortality and is caused by a mutation or deletion of the survival of motor neuron 1 (SMN1) gene, resulting in reduced expression of the ubiquitous SMN protein. Motor neurons (MNs) are particularly impacted by decreased SMN protein levels, and MN loss is the primary phenotypic outcome in SMA patients. SMN deficient MNs show intrinsic deficits in splicing and function, but these defects alone are not sufficient to induce overt MN loss. Our lab has found that astrocytes differentiated from SMA patient-derived induced pluripotent stem cells (iPSCs) secrete high levels of pro inflammatory ligands into their media (ACM) compared to healthy control (HC) astrocytes and that SMA ACM is capable of inducing MN loss. We also found that SMA iPSC-derived microglia display increased reactive morphology and phagocytosis when exposed to SMA ACM compared to HC ACM. We identified CCL5 and IL-1ra as promising targets for reducing the SMA pro-inflammatory astrocytic phenotype and hypothesized that reducing CCL5 while increasing IL-1ra signaling ($IL\text{-}1ra^{inc}/CCL5^{neut}$) would ameliorate astrocyte-driven glial activation and MN loss in SMA. We found an overall reduction in SMA astrocytic pro- inflammatory phenotype after $IL\text{-}1ra^{inc}/CCL5^{neut}$ treatment. SMA $IL\text{-}1ra^{inc}/CCL5^{neut}$ ACM reduced microglia priming and phagocytosis in both monocultures and co-cultures with SMA MNs. $IL\text{-}1ra^{inc}/CCL5^{neut}$ ACM treated SMA MNs also showed an improvement in calcium function in mono- and co-culture conditions compared to SMA ACM treated MNs. Together, these data support the idea that astrocyte-targeted $IL\text{-}1ra^{inc}/CCL5^{neut}$ treatments decrease astrocyte-mediated microglial activation and MN loss in SMA and may allow for an extended therapeutic window. This hypothesis is now being examined in vivo using a gene therapy approach to target IL-1ra overexpression and CCL5 knockdown specifically to astrocytes in the SMNΔ7 mouse model of SMA.

12 The AP-1 clathrin adaptor protein complex coordinates human cortical tissue morphogenesis and forebrain patterning

Amber E. Carleton, Sicong Wang, Mara C. Duncan and Kenichiro Taniguchi

acarleton@mcw.edu

Several variants in genes encoding subunits of the AP-1 clathrin adaptor protein complex, a critical regulator of clathrin-dependent membrane trafficking, have been linked to rare human neurodevelopmental disorders associated with cortical malformation. Although some clinical manifestations are thought to be caused by AP-1 dependent effects on the localization of copper transporters, sortilins and secretases, the extent to which changes in these cargo contribute to the development of human cortical tissues remains unclear. To gain additional mechanistic insights into the role of AP-1 in human cortical tissues, we generated human cortical organoids (HCO) derived from control human embryonic stem cells (hESC) and from hESC lacking AP-1-γ1, a subunit that plays an integral role in maintaining AP-1 complex structure. Strikingly, in the AP-1-γ1-KO HCO, the apical lumen of the cortical epithelium failed to

properly expand, an apical morphogenesis defect similar to one seen in the AP-1-γ1-KO human epiblast model (Wang *et al.* Science Advances 2021). Additional investigation using single cell transcriptomic analyses of 22-day old control HCO revealed several previously uncharacterized SOX2$^+$ neuroepithelium/neural progenitor subpopulations, including rostral and caudal telencephalon-like cells labeled by *FGF8* and *EMX2*, respectively. Strikingly, in the KO HCO, there is a marked increase in the *EMX2*$^+$ caudal telencephalic population, while *FGF8*$^+$ rostral population is largely diminished. Because the formation of neuronal (e.g., *TBR1*$^+$, *NEUROD1*$^+$, *MAP2*$^+$) and other cell types remained largely intact in the KO HCO, these results suggest an intriguing role of AP-1-γ1 in human forebrain patterning. These studies present the first detailed analysis of the role of the AP-1 complex during human cortical development and provide new insights into potential mechanisms underlying the AP-1 associated neural disorders. Funding: This work was supported by NIH grants R01-HD102496 and R01-GM129255; MCW CBNA Start-up funds.

13 Identifying novel cardiac regeneration enhancers by utilizing computational analyses and transgenic assays

Ian J. Begeman1, Steffani Manna1, Shikha Vashisht2, Cecilia Winata2, Junsu Kang1
1 Department of Cell and Regenerative Biology, School of Medicine and Public Health, University of Wisconsin–Madison
2 Laboratory of Zebrafish Developmental Genomics, International Institute of Molecular and Cell Biology in Warsaw
begeman@wisc.edu

Heart regeneration relies on the reconstruction of gene regulatory networks (GRNs) in response to cardiac injury, which is orchestrated by tissue regeneration enhancer elements (TREEs). Identifying groups of TREEs exhibiting similar features will provide a base for elucidating GRNs that control heart regeneration. We previously dissected a cardiac regeneration enhancer in zebrafish to determine the regulatory mechanisms governing heart regeneration. The cardiac leptin b regeneration enhancer (cLEN) exhibits injury-inducible activity near the wound in the heart, which is conferred by multiple injury-activated regulatory elements distributed throughout the enhancer. Our analysis also found that cardiac regeneration enhancers are not only activated by injury, but are also actively repressed in the absence of injury, demonstrating dual regulatory mechanisms of cardiac TREEs. Our extensive transgenic assays identified a short 22-bp DNA element containing a key repressive element responsible for maintaining the inactivation of cLEN in uninjured hearts. To uncover a group of TREEs similar to cLEN present in the genomes of zebrafish, mice, and humans, we devised a strategy to identify cLEN-like enhancer candidates by analyzing sequence similarity, evolutionary conservation, target gene functionality, and epigenomic and transcriptomic profiles. Transgenic assays confirmed that multiple predicted enhancers in the zebrafish genome exhibit injury-dependent activation in regenerating hearts. Identifying additional regeneration enhancers across species will expand our understanding of the transcriptional mechanisms underlying heart regeneration and lead to the identification of potential targets for improving heart repair.

14 Co-activation of canonical Wnt and Notch signaling in human PSC-derived endothelial progenitors induces blood-brain barrier properties

Sarah M Boutom[1], Yunfeng Ding[2], Benjamin D. Gastfriend[2], Sean P Palecek[2], Eric V Shusta[2]
boutom@wisc.edu

Induction of blood-brain barrier (BBB) properties in central nervous system (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 derived from human pluripotent stem cells (hPSCs) can be used to study human BBB development and cerebrovascular disease. Human-derived *in vitro* models can be used to probe the relative importance of specific signaling pathways, including Wnt/β-catenin and Notch, on induction of BBB properties in naïve endothelium. These signaling pathways are typically attributed to ligands derived from neural progenitors and pericytes in the developing CNS. We differentiated endothelial progenitor cells (EPCs) expressing both CD31 and CD34 from hPSCs and treated the hPSC-EPCs with CHIR99021 to activate the Wnt/β-catenin signaling cascade and genetic overexpression of the Notch1 intracellular domain (*N1ICD*) to simulate signaling through the Notch1 receptor. We find that co-activation of Wnt/β-catenin and Notch1 signaling resulted in simultaneous upregulation of Glut-1, a BBB-enriched glucose transporter, and decreased expression of both PLVAP and caveolin-1, two vesicular transcytosis-associated proteins. These findings suggest that the combination of these two signaling inputs yields induction of important barrier properties, including expression of a nutrient transporter and global reduction of transcytosis-associated protein expression. Future studies will use transcriptomic analysis to investigate if this signaling combination is sufficient for induction of other major BBB properties in hPSC-derived endothelial cells. The identification of pathways with potential importance for developmental specification of BBB properties in humans will also advance efforts to model the human BBB *in vitro* for the study of CNS drug delivery and neurovascular disease.

15 Regulation of adult neural stem cells by G protein-coupled receptor Gpr62

Elizabeth D. Clawson[1,2,3,4,5], Daniel Z. Radecki, PhD[1,2,3,4], Jayshree Samanta., MBBS, PhD[1,2,3,4][1]University of Wisconsin-Madison, [2]School of Veterinary Medicine, [3]Department of Comparative Biosciences, [4]Stem Cell and Regenerative Medicine Center, [5]Molecular and Cellular Pharmacology Program

elizabeth.clawsom@wisc.edu

An important goal in the treatment of demyelinating diseases such as Multiple Sclerosis (MS) is to enhance remyelination, which is typically limited in the central nervous system. Remyelination requires activation/expansion of progenitor cells, their recruitment to lesion sites, their differentiation into oligodendrocytes and formation of myelin sheaths around demyelinated axons. A critical bottleneck for successful remyelination is maturation of oligodendrocytes, which is blocked even in the healthy aging brain.

We have identified GPR62, an orphan G protein-coupled receptor (GPCR), as an inhibitor of maturation in oligodendrocytes derived from primary adult neural stem cells. GPR62 belongs to the Class A (Rhodopsin) family of GPCRs with constitutively active cAMP signaling; its endogenous ligand is not known. Interestingly, patients with primary progressive MS have significantly higher levels of autoantibodies against GPR62 compared to those with relapsing remitting MS. However, the functional role of GPR62 in the brain and the downstream signaling pathways activated for remyelination remain unknown. Remarkably, GPR62 is enriched in the white matter but genetic loss of GPR62 does not affect developmental myelination in mice. Our results show that oligodendrocytes generated by primary adult neural stem cells from GPR62 knockout mice produce a highly complex morphology with significantly greater branching and higher number of myelin expressing processes suggesting that these oligodendrocytes may be able to myelinate many more axons than the wildtype oligodendrocytes. Thus, our studies provide new insights into mechanisms driving remyelination and may lead to the identification

of a novel therapeutic target for primary progressive MS which is not treatable by currently available MS drugs.

16 Histone demethylase control of primordial germ cell specification

Harshini Cormaty, Caleb Dillingham, Ellen Morgan
cormaty@wisc.edu

During embryonic development approximately 40 cells are designated as Primordial Germ Cells (PGCs) and are destined to become sperm or egg. These cells undergo massive epigenetic remodeling, which is not seen in the surrounding somatic cells. The repressive histone modification H3K9me2 is depleted during PGC specification. In parallel, DNA is demethylated in two waves: a global erasure followed by a loci specific depletion. The only locations to escape this first wave of global erasure are a few germ cell specifying genes, parental imprinting loci, and retroviral elements. How these loci are protected during this process is still unknown. The enzymes that catalyze this epigenetic remodeling are H3K9me2 specific histone demethylases, KDM3A and KDM3B, and the DNA demethylases, TET enzymes. Our prior work from somatic cell reprogramming to induced pluripotent stem cells, indicated that there is coordinated removal of H3K9me2 and DNA methylation. We hypothesize that a similar mechanism is at play during germ cell development. Therefore, we have established an in vitro differentiation model of embryonic stem cells to primordial germ cell like cells (PGCLCs) in which we can rapidly eliminate the KDM3 proteins using a "degron" tag. Using this tool, we have found that complete removal of the KDM3 proteins compromises PGCLC development. The results from this work will inform germ cell specification and fertility.

17 Neurotransmitter gamma aminobutyric acid (GABA) regulates B cell lineage commitment of hematopoietic stem cells via a feedback loop

Cesi Deng, Adedamola Elujoba-Brindenstine, Casey J. Ostheimer, Owen J. Tamplin
cesi.deng@wisc.edu

The nervous system regulates hematopoiesis through different mechanisms, including neurotransmitters and direct innervation of the bone marrow niche. Understanding the crosstalk of neural and hematopoietic systems will open the possibility of using neuroregulators to modulate hematopoiesis. Previously, we established that the neurotransmitter gamma aminobutyric acid (GABA), and its receptor GABBR1, has a role in hematopoietic stem and progenitor cell (HSPC) function and differentiation. We showed that B cells were the major source of GABA in vivo in the bone marrow. We have now confirmed that GABA is produced autonomously during B cell differentiation in co-cultures with OP9 stromal cells, and increasing GABA levels correlates with increasing numbers of B cells. We hypothesize that B cell-derived GABA regulates B-cell lineage commitment of HSPCs via a feedback loop. To test this hypothesis, we treated HSPCs with different concentrations of GABBR1 agonists in B cell differentiation co-cultures. We found low doses promoted and high doses inhibited HSPC to B cell differentiation, a pattern that suggests GABA regulates HSPC differentiation in a dose-dependent manner. To test the role of GABA production in B cells during lineage commitment, we generated two mutant lines where glutamate decarboxylase1/2 (GAD1/2) enzymes were deleted specifically in B lineage progenitors (Mb1-Cre;Gad1/2-fl/fl and Cd19-Cre;Gad1/2-fl/fl). Although we observed no change in the hematopoietic profiles of the two mutant lines in vivo, we found that in vitro differentiation of mutant HSPCs impaired B cell production. These findings indicated an intrinsic role for GABA production in B lineages during HSPC

differentiation that may be masked in vivo by alternate sources of GABA in the bone marrow microenvironment. This research demonstrated that B cell-derived GABA regulates HSPC activity and suggests that GABA might be repurposed to modulate B cell numbers and improve immune function.

18 Multi-omic Characterization of Human Pluripotent Stem Cell-derived Cardiomyocyte Maturation during Long-term Culture

Austin K. Feeney**,** Aaron D. Simmons, Elizabeth F. Bayne, Melissa R. Pergande, Yanlong Zhu, Timothy J. Kamp, Ying Ge, and Sean P. Palecek

afeeney@wisc.edu

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) offer the ability to enhance drug discovery and restore damaged heart tissue through cellular therapy. However, generating hPSC-CMs with a mature phenotype remains a challenge. Although several methods to enhance hPSC-CM maturation have previously been investigated, hPSC-CM immaturity continues to limit in vitro and in vivo utility. Interestingly, few studies have sought to characterize hPSC-CM maturation at the systems biology level. To gain a better understanding of the molecular dynamics which occur during maturation, we performed an integrative, multi-omic profiling of hPSC-CMs matured via extended culture through 190 days. We identified significant changes in 305/948 intracellular metabolites, 1,158/3,556 proteins, and 12,813/23,309 transcripts. Additionally, we have characterized the dynamic sarcomeric proteoform and extracellular metabolite profiles. Interestingly, we identified different timescales in which various aspects of CM maturation occur. For example, substantial sarcomeric maturation occurs within 60 days and plateaus by 100 days (evidenced by transcript and proteoform switches). However, metabolic maturation continually increases throughout the entire 190 days, with glucose utilization and lactate secretion declining while transcripts related to oxidative metabolism continually increase. Moreover, metabolic pathway analysis demonstrates an increase in fatty acid oxidation from day 30 to 190. In addition to temporal waves of maturation, we have identified putative novel maturation markers and compared them to other studies of hPSC-CM and in vivo maturation. These findings will serve as a benchmark for future studies accelerating hPSC-CM maturation to unlock their full potential for drug discovery, disease modeling, and heart repair.

19 UW-Madison Stem Cell and Regenerative Medicine Center symposium abstract 2023

***Rut Gabarro-Solanas*[1,2], Amarbayasgalan Davaatseren[1], Tatjana Kepcija[1], Ivan CrespoEnriquez[1] and Noelia Urban[1]*

[1]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria.

[2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, A-1030, Vienna, Austria. *Correspondence: noelia.urban@imba.oeaw.ac.at

rut.gabarro.solanas@imba.ac.at

Adult neural stem cells (NSCs) in the dentate gyrus (DG) generate new neurons throughout life that integrate into existing hippocampal circuits to modulate memory and emotions. NSCs are mostly quiescent, and their activation is coupled to exhaustion because they rarely self-renew. Despite their ability to return to quiescence after activation, NSC numbers and activity decline with age, resulting in a sharp decrease in neurogenesis in old animals. Intermittent fasting (IF) is seen as a promising strategy to slow down aging and is considered to increase the number of

newly born cells in the DG of adult mice. However, whether IF directly regulates NSCs has not been investigated. Here, we aimed at elucidating the effects of IF on NSC quiescence and maintenance. To follow NSCs and their progeny, we performed lineage tracing in GlastCreERT2;RYFP mice in which we could permanently label NSCs upon tamoxifen induction; and combined it with thymidine analogue retention experiments to explore NSC transitions between quiescence and activation. We found that 1 month of every-other-day IF did not affect NSC proliferation, quiescence/activation transitions nor maintenance. We then fasted the mice for three months and saw that NSCs were resilient to IF, suggesting that the previously described increase in neurogenesis originated in later stages of the neurogenic lineage. To our surprise, when we quantified the number of intermediate progenitor cells, neuroblasts and newly born neurons, we observed that IF did not promote neurogenesis nor could halt aging in the DG. Since these data contradicted previous reports, we explored the influence of multiple variables such as sex, mouse strain, labelling method, tamoxifen administration and diet length on the effects of IF. We consistently found that IF was unable to promote the generation of new neurons, suggesting that IF is not a reliable strategy to increase adult neurogenesis.

20 Elevated levels of FMRP-target MAP1B impair neuronal development and social behaviors via autophagy pathway

Yu Guo1,2, Minjie Shen1,2, Qiping Dong1, Natasha M. Méndez-Albelo1,2, Jonathan Le1,2, Meng Li1,2,#, Sabrina X. Huang1,2, Ezra D. Jarzembowski1,2, Keegan A. Schoeller1,2, Michael E. Stockton1,2, Carissa L. Sirois1,2, Vanessa L. Horner3,4, Andre M. M. Sousa1,2, Yu Gao1,2, Birth Defects Research Laboratory5, Jon E. Levine2,6, Daifeng Wang1,7, Qiang Chang1,8,9, and Xinyu Zhao1,2*
yguo275@wsic.edu

Functional deficiency of fragile X messenger ribonucleoprotein 1 (FMR1) protein (FMRP) leads to fragile X syndrome (FXS). FXS is the most common heritable cause of intellectual disability but also the largest single genetic contributor to autism spectrum disorder (ASD). FMRP binds many mRNA targets in the brain. However, very few FMRP targets have been investigated in human neurons and the contribution of these targets to fragile X syndrome (FXS) and related autism spectrum disorder (ASD) remain unclear. In this study, we provide evidence to show that FMRP controls microtubule-associated protein 1B (MAP1B) level in developing neurons and such regulation is conserved in primates by using ex vivo human and rhesus macaque brain slice culture and FXS patient-derived neurons. Targeted *MAP1B* gene activation in healthy human neurons or *MAP1B* gene triplication in ASD patient-derived neurons inhibit morphological and physiological maturation. Activation of *Map1b* in mouse prefrontal cortex excitatory neurons impairs social behaviors. We show that elevated MAP1B sequesters components of autophagy and reduces autophagosome formation. Both MAP1B knockdown and autophagy activation rescue deficits of both ASD and FXS patients' neurons and FMRP-deficient neurons in ex vivo human brain tissue. Our study demonstrates conserved FMRP regulation of MAP1B in primate neurons and establishes a causal link between MAP1B elevation and deficits of FXS and ASD.

21 Elucidating the influence of microglia on retinal ganglion cells in a human pluripotent stem cell model

Jade Harkin1,2, Cátia Gomes2,5,6, Kiersten Pena3, Sailee S. Lavekar2,3, Kang-Chieh Huang2,3, Jason S. Meyer1,2,5,6

1Department of Pharmacology and Toxicology, 2Stark Neurosciences Research Institute, 3Department of Biology, 4Department of Medicine, 5Department of Ophthalmology, Glick Eye Institute,6Department of Medical and Molecular Genetics, Indiana University School of Medicine

harkinj@iu.edu

Glaucoma is the leading cause of irreversible blindness worldwide, affecting approximately 80 million people. In animal models of glaucoma, microglial activation has been associated with changes in morphology and proliferation, as well as the release of large amounts of inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin IL-1β, and IL-6, which contribute to the neurodegeneration of retinal ganglion cells (RGCs). Due to major differences identified between rodent microglia and RGCs compared to humans, there is a critical need for novel human models that can be used to explore the cellular crosstalk between human microglia and RGCs and the role of this interaction in neurodegenerative diseases such as glaucoma.

In the current study, we differentiated both microglia-like cells (MGLs) and RGCs from human induced pluripotent stem cells (iPSCs). Microglia activation was then induced using Lipopolysaccharide (LPS) and confirmed using morphological analysis, immunostaining, and cytokine/chemokine profile screening. Healthy and LPS-activated microglia were then co-cultured with RGCs for up to 3 weeks and the effects of microglia upon RGCs were assessed in both direct and non-direct co-cultures using measurements of soma size and neurite complexity.

Results indicate that after treatment with LPS, iPSC-derived microglia exhibit more round, amoeboid morphologies, increased expression of MHC-II and release inflammatory cytokines including IL-6, IL-8, IL-1B and TNF-a. Additionally, when co-cultured with RGCs, LPS-activated microglia reduced RGC neurite complexity and soma size compared to healthy microglia, suggesting a contribution of microglia activation to RGC neurodegenerative features.

Taken together, the current study establishes for the first time a human model system that allows us to examine the cellular interactions between human microglia and RGCs and study microglial contributions to RGC neurodegeneration.

22 CARDIAC TISSUE ENGINEERING WITH GENE-EDITED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES ENABLES DETECTION OF EARLY-STAGE MECHANISMS THAT GOVERN HYPERTROPHIC CARDIOMYOPATHY

Jeanne Hsieh and Brenda M. Ogle

Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455 USA

hsieh125@umn.edu

Hypertrophic cardiomyopathy (HCM) is a common inherited heart disease, affecting 1:500-200 people globally. As HCM-causing mutations mainly exist in the gene loci encoding sarcomeric proteins, cardiac functions are directly disturbed by this disease. Further, HCM is associated with sudden cardiac death in young people and competitive athletes, but a detailed mechanistic understanding of HCM, especially at the early stages of disease progression, is limited. To investigate early-stage HCM triggers and potential strategies to delay or prevent the disease onset and progression, we evaluated the impact of myosin heavy chain (MHC) mutations on HCM pathogenicity in a 2D culture system and an engineered heart tissue (EHT) model by comparing mutant hiPSC-derived cardiomyocytes (hiPSC-CMs) with its isogenic control at

multiple time points. In 2D, we verified gene-edited hiPSC-CMs with MYH7 R723C and MYH6 R725C dual mutations exhibited HCM phenotypes including hypertrophy, multinucleation, altered calcium handling, and irregular beating. Through bulk RNAseq analysis, we identified early extracellular matrix (ECM) changes that precede later-stage physiologic defects associated with HCM. In 3D, fibrin-based EHTs with hiPSC-CMs and fibroblasts were generated to study cardiac function changes caused by HCM mutations. Intercellular communication between hiPSC-CMs and fibroblasts was identified as the key to supporting the cardiac function of MHC-mutant EHTs. Contraction force and TGF-beta 1 secretion were significantly increased in mutant EHTs, and after blocking TGF-beta 1 signaling, the contraction force of mutant EHTs returned to a level comparable to control EHTs. The result of our 2D study is the first to link ECM dynamics with HCM onset. The tissue-level 3D model further enabled the study of the interplay between hiPSC-CMs and fibroblasts in the context of HCM and suggested hiPSC-CMs impact on fibroblasts very soon after specification. Altogether, these findings lay the foundation for novel strategies to treat HCM at the early stages of the disease.

23 The Effects of Propofol on Barrier Integrity in a Human Stem Cell Derived Blood-Brain Barrier Model

***Jason M. Hughes*[1,2]**, Olivia R. Neese[1], Kirsten A. Lewis[1], Scott G. Canfield[1]

[1]Department of Anatomy, Cell Biology, and Physiology, Indiana University School of Medicine–Terre Haute, [2]Department of Biology, Indiana State University

Kimberly.Strain@indstate.edu

According to the World Health Organization, over 230 million major surgical procedures are carried out under anesthesia each year. Numerous studies have shown anesthesia at both sustained and multiple exposures may result in neurodegenerative changes. A portion of these studies have demonstrated that anesthetic exposure induced blood-brain barrier (BBB) dysfunction, however, the effects of anesthesia on the human BBB are still unknown. The BBB consists of specialized brain microvascular endothelial cells (BMECs) supported by astrocytes, neurons, and pericytes and is vital in protecting the brain parenchyma from the surrounding micro-vasculature. In this study we utilized BMECs derived from human induced pluripotent stem cells (iPSCs) to assess the effects of an anesthetic, propofol, on barrier integrity. BMECs were treated with different concentrations of propofol for various durations and barrier properties were monitored post-exposure. Barrier tightness was determined by trans-endothelial electrical resistance (TEER) and fluorescein permeability. Tight junction protein localization and expression were assessed by immunocytochemistry and western blot, respectively. Upon treatment with propofol, iPSC-derived BMECs displayed diminished TEER and elevated sodium fluorescein permeability when compared to non-treatment BMECs, implicating a loss in barrier integrity. Immunocytochemistry indicated a delocalization of Occludin and western blot data displayed an overall decrease in protein expression of Occludin. Additionally, propofol induced an increase in MMP-2 activity, detected by ELISA. Treatment with GM6001, a general MMP inhibitor, partially restored barrier properties when administered prior to exposure to propofol. A further understanding of the cellular mechanisms of propofol induced BBB damage may unveil novel therapeutic targets to improve anesthesia safety.

24 ***Cecilia E. Lekpor[1,3],*** Adriana Harbuzariu[2], Kwadwo A. Kusi[1], Adel Driss[3], Michael D. Wilson[1], Andrew A. Adjei[1], Wesley Solomon,[3] Jonathan K. Stiles[3]
[1]University of Ghana, Accra, Ghana, [2]Korle-Bu Teaching Hospital, [3] Morehouse School of Medicine, Atlanta, GA, United States.
cbotchway@msm.edu

Sickle cell disease (SCD) overlaps *Plasmodium falciparum* (*Pf*) infections in sub-Saharan Africa. In Ghana, 2% of newborns are affected by SCD annually and 30% have sickle cell trait (SCT). SCD individuals may present with severe malaria but SCTs are protected against lethal outcomes. The mechanisms mediating resistance of SCT individuals against human cerebral malaria (HCM) remain unclear. We propose that reprogramming patient-derived adult somatic cells (from skin, blood, or urine) into induced pluripotent stem cells (iPSC) will enable development of patient specific iPSCs for tissue engineering. We hypothesize that urine mesenchymal cells, from children with SCD, SCT and HbAA (10/each group) could be transformed into iPSC's for studying correlation between the mutations and host inflammatory responses to *Pf* infection. Clinical data from children (3-8 y) with SCT and SCD presenting at the Child Health Department (CHD) of Korle-Bu Teaching Hospital (KBTH) in Accra, Ghana were documented (clinic visits, parasitemia, hematological parameters, hemoglobin status, brain injury biomarker levels, and complications). Urine and blood samples from SCT (HbAC, HbAS) and HbAA controls were collected and cultured in defined medium for 2 weeks after which mesenchymal stem cells (UDC) were isolated. Frequency of severe malaria episodes were also compared among normal HbAA and SCD children. UDCs were phenotyped by flow cytometry and cell immunofluorescence (IF). Malaria susceptible and resistant biomarkers were also assessed. Children with SCT had lower *Pf* parasitemia, and number of severe malaria episodes compared with SCD and HbAA. Mesenchymal stem cells (CD73+/CD146+/CD105+) as well as rare smooth muscle cells (SMA+/calponin+) cells at more than 30% confluence were expressed and UDCs stored in our mini repository.

In conclusion, we establish a mini repository of UDC lines to be used to generate tissue organoids for future studies on mechanisms mediating resistance or susceptibility to severe malaria.

Key Words: Human Cerebral malarial; Haemoglobinopathies; Repository

25 POTENTIAL ROLE OF BRIDGING INTEGRATOR PROTEIN 3 (BIN3) IN PARKINSON'S DISEASE

Sakthikumar Mathivanan1,2, Yunlong Tao1,2, Beatrice Weykopf1,3,4, Emily Abella2, Andy Peterson2, Zechuan Lin1,3,4, Zhixiang Liao1,3,4, Jacob Parker1,3,4, Tao Wang1,3,4, Xianjun Dong1,3,4, Joshua Z. Levin1,5, Mel Feany1,6, Clemens Scherzer1,3,4 and Su-Chun Zhang1,2,7*.
1Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA
2Waisman Center, University of Wisconsin-Madison, Madison, WI 53705, USA; Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53705, USA
3Center for Advanced Parkinson Research, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
4Precision Neurology Program of Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
5Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, 02142, USA
6Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
7Program in Neuroscience & Behavioral Disorders, Duke-NUS Medical School, 169857 Singapore, Singapore.
smathivanan@wisc.edu

Genome Wide Association Studies (GWAS) in Parkinson's Disease (PD) patents have identified numerous non-coding variants. Using expression Quantitative Trait Locus (eQTL) analyses of

hundreds of thousands of single brain cells in the Parkinson Brain Atlas intiative and in 1,170 human brains, we identified (Bridging Integrator Protein 3) as an effect gene regulated by a GWAS peak on Chromosome 8. This raises the possibility of BIN3 involvement in PD pathogenesis.
By using CRISPR cas9 gene editing technology, we generated BIN3 Knockout (KO) and Over Expression (OE) human pluripotent stem cell (hPSC) lines. In the BIN3 KO hPSC-derived midbrain dopamine neurons, there is a substantial increase in alpha-synuclein and serine 129 phosphorylated alpha-synuclein. Western blotting showed decreased levels of proteins related to the autophagosomal-lysosomal system, including P62, LAMP1 and LC3BII, suggesting that accumulation of alpha-synuclein may be due to impaired degradation. Indeed, the BIN3 KO cells display enlarged lysosomes, as indicated by lysotracker red. Furthermore, BIN3 KO neurons show reduced endocytosis, especially when the neurons were stimulated, indicated by the uptake of a fluorescent dye. These results suggest that BIN3 plays a role in regulating vesicular trafficking and alteration of BIN3 (e.g., KO) impairs the vesicular trafficking, which may impair dopamine neuron function and result in degeneration.

26 Multicellular Modeling of Human Induced Pluripotent Stem Cell Colonies Informs Control over NANOG and GATA6 Phenotypes

Eunbi Park
epark90@gatech.edu

The early differentiation of human induced pluripotent stem cell (hiPSC) colonies involve multicellular pattern formation based on collective intercellular communication within local and long distances and symmetry breaking. However, it is unclear which mechanisms take priority in context-specific situations, a necessity for further hiPSC-derived tissue engineering. Prior work reported intracellular and intercellular regulation of the FGF/ERK pathway in the mouse blastocyst as a driver of differentiation and self-organization. We analyzed the role of this pathway during the initial loss of pluripotency with genetically engineered hiPSCs at different scales of two- and three-dimensions. In order to derive features associated with a network model of NANOG/GATA6 cross-regulation, we imaged and quantified immunofluorescence microscopy single-cell multivariate data of the expression levels of the proteins. Inhibition studies of the GATA6-inducible hiPSC colonies undergoing differentiation perturbed cell proliferation, distribution of cell states, and spatial organization with FGFR inhibitor PD173074 and MEK inhibitor U0126. In both 2D and 3D settings, perturbations in ERK phosphorylation sustained NANOG high cells after inducing two days of differentiation, whereas FGFR inhibition led to opposite phenotypes. Such perturbations yielded different trends in GATA6 phenotypes at each scale, thereby elucidating context-specific characteristics. A NetLogo agent-based 2D model developed with rules of directed migration, proliferation, and differentiation yielded the experimentally-observed Turing-like contorted patterns of NANOG and GATA6 expression under specific parameter settings. We also developed a general-purpose pipeline that uses topological data analysis (TDA) that quantified differences in the spatial organization of hiPSCs based on different biological markers and experimental conditions. These findings propose that the strategies of quantifying spatial organization and modeling and testing engineered hiPSCs in 2D and 3D colonies are tractable alternative approaches to embryonic systems for predicting and identifying modes of intercellular communication that determine cell fates, directing hiPSCs into differentiated lineages.

27 mTOR Independent Roles of TSC2 in RhoA-mediated Axon Guidance and Growth Cone Collapse

Austin Pier, Gomez Lab, UW-Madison
pier2@wisc.edu

Tuberous Sclerosis Complex (TSC) is an autosomal dominant neurodevelopmental disorder that affects 1 in 6000 individuals. TSC is caused by heterozygous mutations to either TSC1 or TSC2, resulting in the formation of benign tumors in multiple organ systems, including the CNS. A majority of TSC patients develop seizures, while over half of TSC patients present with mild to severe cognitive impairment, autism spectrum disorders, and other neurological deficits. In past clinical work, most TSC symptoms are explained by the presence of cortical tumors, which are attributed to increased protein synthesis caused by the loss of TSC1-TSC2 inhibition of mTOR-mediated protein synthesis. Interestingly, multiple studies found a poor correlation between cortical tuber number, size, and presence and severity of TSC symptoms, indicating alternative mechanisms. In support of alternative disease-causing mechanisms, a few studies utilizing diffusion tensor imaging in TSC patients found significant abnormalities in neuronal connectivity, suggesting patients with TSC suffer from defects in neural network wiring. These human studies are consistent with heterozygous TSC2 animal models that have learning and behavioral deficits but lack cortical tumors.

Furthermore, our recently published work using patient-derived human forebrain neurons indicates that patients with TSC2+/- mutations suffer from dramatic defects in RhoA-mediated axon guidance and growth cone collapse downstream of relevant inhibitory guidance cues. Surprisingly, these observed defects appear to be independent of both mTOR pathways. Our primary research objective is to characterize TSC2's mTOR-independent roles in modulating repulsive cue signaling, specifically focusing on its role in regulating RhoA activity, which is required for proper axon pathfinding. In this work, we will determine if TSC2 modulation of RhoA activity depends on TSC2 inhibition of Rheb activity through its GAP domain and determine which downstream effectors of TSC2 are relevant for mTOR-independent regulation of RhoA-mediated axon guidance and growth cone collapse.

28 ER Ca2+ release channels regulate mitochondrial metabolism in human induced pluripotent stem cells

Julius Rönkkö1, Yago Rodriguez1, Tiina Rasila1, Rubén Torregrosa-Muñumer1, Jana Pennonen1, Jouni Kvist1, Emilia Kuuluvainen2,3, Ludo Van Den Bosch4,5, Ville Hietakangas2,3, Geert Bultynck6, Henna Tyynismaa1, Emil Ylikallio1,7+

1University of Helsinki, Helsinki, 00290,Finland
[2] Molecular and Integrative Bioscience Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, 00790, Finland
[3] Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, 00790, Finland
4 Department of Neurosciences, Experimental Neurology and Leuven Brain Institute, KU Leuven – University of Leuven, 3000, Leuven, Belgium
5 VIB Center for Brain & Disease Research, Laboratory of Neurobiology, 3000, Leuven, Belgium
6 KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut, Leuven, 3000, Belgium
7 Clinical Neurosciences, Neurology, University of Helsinki and Helsinki University Hospital, Helsinki, 00290, Finland
julus.ronkko@helsinki.fi

Charcot-Marie-Tooth disease (CMT) is a group of inherited peripheral neuropathies. One of the causes of demyelinating type of CMT are dominant variants in inositol 1,4,5-trisphosphate receptor (IP3R) type 3. IP3Rs are the main Ca2+ release channels in ER, transferring Ca2+ to

cytosol and mitochondria. There are three IP3R isoforms, encoded by three distinct genes and with unique cellular localization and tissue-specific expression. To understand the pathogenesis of CMT, we developed induced pluripotent stem cell (iPSCs) models to elucidate the cell type-specific functions of IP3 receptors.

We generated single and triple knockout (TKO) iPSC cell lines of IP3 receptors using CRISPR/Cas9 and are in process of editing patient specific CMT mutations. We then confirmed the successful editing with RT-qPCR and western blotting, and analyzed the resulting KO lines with pluripotency gene expression panel, embryonic body formation assay, functional Ca2+ imaging, and LC-MS and U-13C labeled glucose fluxomics.

Our results showed that IP3Rs are not essential for iPSC identity and pluripotency, although they regulate mitochondrial metabolism in iPSC, as TKO iPSC exhibited a deficiency in pyruvate utilization via pyruvate dehydrogenase (PDH) and a shift towards pyruvate carboxylase pathway (PC). The loss of IP3Rs resulted in significant alterations in mitochondrial metabolism, which may be relevant for the development of CMT when IP3R3 is defective. Furthermore, we successfully differentiated the IP3R TKO lines to Schwann cells and motor neurons, allowing us to further study the role of IP3Rs in these cell types of disease interest.

This is the first description of complete functional loss of IP3 receptors in human iPSC. Our results show that IP3 receptors regulate stem cell metabolism but are not required for maintenance of pluripotency. The generated iPSC lines provide a robust model for elucidating how IP3R-mediated calcium signaling contributes to peripheral nervous system function.

29 Integrated Proteomics Analysis of *In Vitro* Hypertrophic Cardiomyopathy Models

Kalina J. Rossler, Willem de Lange, Jake A. Melby, Morgan W. Mann, Timothy J. Aballo, Jianhua Zhang, Gina Kim, Elizabeth F. Bayne, Yanlong Zhu, Timothy J. Kamp, J. Carter Ralphe, and Ying Ge
kjreese@wisc.edu

Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in young adults. Nearly 1,500 HCM-related mutations in sarcomeric genes have been identified, but the molecular events leading to the disease phenotype remain largely unknown. While previous studies have focused on functional and transcriptional changes in HCM-specific human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CMs) models, comprehensive characterization of proteome changes in regards to functional phenotype has yet to be explored. Herein, we have generated 2D (monolayer) and 3D (engineered cardiac tissues; ECTs) hiPSC-CM models from family members with no mutation and an HCM-causing β-myosin heavy chain (*MYH7*) R663H mutation to be evaluated via mass spectrometry (MS)-based proteomics. Functional measurements showed HCM models displayed aberrant calcium handling and elongated twitch force curves to the control group. Intact sarcomere protein analysis revealed differential expression of myosin light chain 2 (MLC-2) isoforms in monolayer cultures ($p<0.01$), mirroring the upregulation of the ventricular isoform observed for HCM manifestation*in vivo*. Furthermore, our HCM ECT models uncovered significant decreases in total phosphorylation for contractile proteins MLC-2v and alpha-tropomyosin compared to controls. Global bottom-up proteomics data determined that extracellular matrix (ECM) proteins such as collagen I were downregulated in both disease models. Along with key hypertrophic factors, proteins involved in glucose metabolism were upregulated in the HCM samples. These results revealed altered function and proteoform landscape in both monolayer and ECT HCM models. Indeed, our work to integrate functional and molecular parameters in “early stage” HCM constructs provides mechanistic insight into the progression of cardiac disease.

30 HUMAN STEM CELL-BASED APPROACHES TO STUDY GENETIC RISK FOR CORONARY ARTERY DISEASE

***Elsa Salido*,** Carolina de Medeiros Vieira, Huan Yang, and Valentina Lo Sardo
elsasy@live.com

Coronary Artery Disease (CAD) is the most common type of heart disease, and the primary cause of death worldwide. Several genetic risk factors have been identified through Genome Wide Association Studies (GWAS), but their function is largely unclear. Among these, the most impactful is the 9p21.3 CAD risk locus, a 60 kb gene desert region of the human genome, harboring several Single Nucleotide Polymorphisms (SNPs) in high linkage disequilibrium in most ethnicities. Previously, we identified a role for the risk haplotype at 9p21.3 in influencing vascular smooth muscle cells (VSMCs) homeostasis and function. iPSC-derived VSMCs from individuals carrying the risk haplotype at 9p21.3 showed a dedifferentiation phenotype and functional alterations consistent with the role VSMCs have previously been shown to play in early CAD. Specifically, the 9p21.3 CAD Risk haplotype induces downregulation of VSMC markers, increases proliferation rate, and causes loss of adhesion and contraction properties in VSMCs. Deletion of the risk haplotype restores the non-risk phenotype, suggesting a gain of function effect of the risk variants in this locus. Here, we present a single cell transcriptomic analysis of iPSC-derived VSMCs with different genotypes at 9p21.3 to dissect the role of this locus in causing cell state changes in the vascular wall. Analysis of the risk-driven cell state transition and the identification of disease-prone cell subpopulations suggest early pathological alterations acting in the muscle layer of the arteries. This study provides insights into the early stage of CAD pathogenesis driven by the 9p21 risk locus and offers a method to explore the functional impact of human genomic risk regions by using large scale genome editing approaches and single cell transcriptomic.

31 Establishing Methods for Quantitative Analysis of Brain Organoids to Study Neurodevelopmental Disorders

***Soraya O Sandoval*[1,2]**, Xinyu Zhao[1,2]
[1]Neuroscience Training Program, Neuroscience, Madison, WI, [2]Waisman Center, Neuroscience, Madison, WI
sosandoval@wisc.edu

Human induced pluripotent stem cell (iPSC)-derived cortical organoids have become an increasingly promising tool for the study of human neurodevelopment because they resemble human brain formation. Therefore, many of the techniques and quantitative methods used to study embryonic brain tissue have been applied to study organoids. These include cell proliferation, differentiation, and death, as well as lineage specification, morphology, and physiology of putative cortical layers. However, unlike the human brain, organoids display vast heterogeneity within and across PSC lines, differentiation methods, and differentiation batches. A standardized quantitative framework for studying organoids is lacking. Currently, many of the imaging techniques and tools available to assess organoids are time-consuming, prone to bias, and may introduce variability, rendering quantification a challenge. To address this challenge, together with the IDDRC consortium, we have conducted an in-depth analysis of published quantitative methods for brain organoids. We have recommended unbiased methods that can be applied for reproducible quantification of organoids. Finally, using these methods, we have identified neurogenic deficits in organoids differentiated from iPSCs of individuals diagnosed with fragile X syndrome, a pervasive neurodevelopmental disorder. Accurate quantification of

organoids will reduce variability and increase reproducibility across labs, increasing the use of organoids in the study of brain diseases.

32 Bioengineering Morphogenesis of 3D Neuroepithelial Tubes

Frank Seipel, Carlos Marti Figueroa, Gavin Knight, Randolph S. Ashton
Department of Biomedical Engineering, University of Wisconsin-Madison
Wisconsin Institute for Discovery
fseipel@wisc.edu

Three-dimensional organoid systems derived from human pluripotent stem cells (hPSCs) have proven to be a valuable tool for modeling aspects of human central nervous system (CNS) development that cannot captured by 2D cell culture platforms or animal studies. Most human CNS organoid protocols, however, rely on unguided self-organization, resulting in large tissues with multiple polarizing centers and highly variable cytoarchitecture. This is not reflective of *in vivo* development where the CNS arises from a neuroepithelial tube, an early embryonic structure comprised of a singularly polarized tube of neural stem cells. Here, we have developed a method to biomanufacture hPSC-derived, 3D, and singularly polarized neuroepithelial tubes. Microcontact printing is used to restrict the morphology of adherent hPSCs to centimeter long patterns consisting of a series 250-micron diameter circles connected by narrow bridges. This initial geometric restriction causes the tissues to fold in on themselves as they grow and differentiate in neural induction culture. Providing the tissues with an extracellular matrix environment in the form of a Matrigel hydrogel allows them to fold into 3D neuroepithelial tubes. The resulting neuroepithelial tubes accurately recapitulate the morphology of the embryonic neural tube with a single, hollow lumen and apical polarization of N-Cadherin. This method enables derivation of neural organoids with biomimetic morphology, low batch to batch variability, and a physiologically relevant macroscale cytoarchitecture which is lacking in current CNS organoid systems.

This work was supported by NSF CAREER Award 1651645 and NIH/NCATS UG3TR003150 and UH3TR003150

33 Bone Matrix Mineralization and Breast-Cancer Derived Factors Synergistically Regulate Mesenchymal Stem Cell Behavior in Osteogenic Niches

Nicole Sempertegui
nds68@cornell.edu

Bone metastases occur in over 80% of advanced breast cancer patients. They result in significant morbidity and ultimately high mortality, but the underlying mechanisms remain poorly understood. While overt bone metastasis manifests by osteolysis in the clinic, cancer cells initially colonize osteogenic niches in bone. Mesenchymal Stem Cells (MSCs) and their osteoblast derivatives make up the osteogenic niche and regulate bone mineralization homeostasis. Although decreased bone mineral density predicts increased risk of bone metastasis, how the bone extracellular matrix (ECM), which is composed of hydroxyapatite (HA)-embedded collagen I fibrils, regulates MSC phenotype and how these changes in turn impact tumor cells remains poorly understood. To better understand these connections, model systems are needed that allow controlling collagen mineralization for mechanistic studies. Here, I am using biofunctional materials systems to study the hypothesis that loss of collagen mineralization and presence of breast cancer-derived factors reduce MSC differentiation into osteoblasts, while promoting differentiation into tumor-promoting myofibroblasts. Our results show that a mineralized matrix supports MSC osteogenic differentiation, but breast cancer-derived factors reduce this effect. Additionally, loss of matrix mineralization alters MSC matrix

deposition and increases inflammatory cytokine secretion, which in turn increases tumor cell growth. Overall, these results suggest that loss of collagen mineralization and presence of breast cancer-derived factors impact MSC differentiation fate, and that these changes may result in a favorable niche for breast cancer tumor cell outgrowth in bone.

34 Psilocin as a potential therapeutic agent for cognitive decline: evidence from in vitro human astrogliosis

***Leticia Souza[1],[3], *Teresa Pijuan[1],[3],** Natasha Karassina[3], Jolanta Vidugiriene[3], Alexander Sherwood[2], James J. Cali[3], Stevens Rehen[1],[2],[3]

[1] D'Or Institute, Brazil; 2 Usona Institute; [3] Promega Corporation

leticia.souza@promega.com teresa.pijuan@promega.com

Cognitive decline is a phenomenon often associated with aging, degenerative diseases, sepsis, long ICU stays, trauma, or surgery. Astrogliosis is a prominent feature of brain pathology, distinguished by significant morphological and functional alterations in astrocytes and the secretion of pro- inflammatory cytokines, including interleukin-6 (IL-6). Astrogliosis contributes to cognitive decline in both rodents and humans. Recently, we established a cellular model that recapitulates canonical astrogliosis events in vitro (Trindade et al, 2020). Here, we used Lumit assays to measure cytokine levels in iPS-derived human astrocytes. We found that astrogliosis increased the release of cytokines, such as IL-10, and IL-1β, by at least three-fold, while IL-6 levels increased by five times in human astrocytes. Interestingly, cytokine levels remained elevated even after TNF-alpha stimulation ceased. Thus, we tested the hypothesis that psilocin, a psychedelic tryptamine present in hallucinogenic mushrooms, could reduce astrogliosis-related inflammation. Our results showed that 100 nM psilocin exposure provided a two-fold reduction in the continuous release of IL-6 from TNF-alpha-stimulated astrocytes, without affecting other cytokines. In the immune system, a reduction of IL-6 can be achieved by increasing NAD+ levels in peripheral blood mononuclear cells (Zhou, 2020). We hypothesized a similar correlation between IL-6 decrease and NAD+ increase in human brain cells exposed to psilocin. Human brain spheroids, containing neural stem cells, immature astrocytes, and neurons, were exposed to psilocin. This psychedelic tryptamine increased NAD+ levels by 1.4-fold. Our findings suggest that psilocin may reduce astrogliosis- related IL-6 levels and increase the production of NAD+ in human brain cells. We propose that psilocin should be further studied as a therapeutic agent to reduce cognitive decline caused by multiple factors.

*L.S. and T.P. receive fellowships from the Brazilian Pioneer Science Initiative/D'Or Institute. They contributed equally to this work.

35 Defining a Rejuvenation Signature in Transcription Factor Mediated Cellular Reprogramming

Andrew I. Tak[1,2], Spencer Halberg[2], Sushmita Roy[2,3], Rupa Sridharan[2,4]

[1]Molecular and Cellular Pharmacology Training Program, School of Medicine and Public Health, University of Wisconsin – Madison

[2]Wisconsin Institute for Discovery, University of University of Wisconsin – Madison [3]Department of Biomedical Informatics, University of Wisconsin – Madison

[4]Department of Cell and Regenerative Biology, University of Wisconsin – Madison

aitak@wisc.edu

Aging is a natural process that occurs in all organisms. In humans, advanced age is correlated with a higher incidence of debilitating and degenerative diseases. Cellular hallmarks of aging include the loss of heterochromatin and an increase in DNA damage markers and mitochondrial reactive oxygen species. Remarkably, these hallmarks of aging are reset when somatic cells are

reprogrammed into induced pluripotent stem cells (iPSCs) by the overexpression of the Yamanaka transcription factors. Interestingly, stable iPSC formation is not required for aging reversal, indicating that reprogramming and rejuvenation are independent programs. Therefore, we will uncouple a novel gene regulatory network of rejuvenation from that of cellular reprogramming. To generate such a gene regulatory network we will compare the gene expression and chromatin accessibility profiles of young and old cells at the single-cell level as they reprogram using advanced computational inference algorithms.

36 Generation of brain microvascular endothelial cells through transcription factor-based cell fate engineering

Soniya Tamhankar1, Yunfeng Ding1, Eric V. Shusta1,2 and Sean P. Palecek1
1Department of Chemical and Biological Engineering, University of Wisconsin – Madison, Madison, WI, USA
2Department of Neurological Surgery, University of Wisconsin – Madison, Madison, WI, USA
soniya.tamhankar@wisc.edu

The blood-brain barrier (BBB) is a selective barrier that regulates the transport of molecules between the bloodstream and the central nervous system (CNS). The development of in vitro models of the BBB using human induced pluripotent stem cells (hiPSCs)-derived brain microvascular endothelial cells (BMECs) offers a promising approach to study BBB biology and drug delivery to the CNS. Transcription factors are key regulators of cell fate and differentiation. Studies have shown that they can be used to transdifferentiate cells between lineages, reprogram somatic cells to pluripotency and differentiate stem cells. We aim to develop a protocol to generate BMEC-like cells through transcription factor (TF)-based cell fate engineering. Using publicly available scRNA-seq datasets for endothelial cells (ECs) from various organs in human and mice, we identified TFs that are differentially expressed in brain ECs. We overexpressed six of the candidate TFs by lentivirus transduction in hiPSC-derived endothelial progenitor cells (EPCs), differentiated using a previously established method. We screened these TFs on EPCs to measure changes in the gene expression of BBB-related transcripts, and observed that some TFs improve the expression of junctional genes, while some TFs reduce the expression of endocytosis-related genes, which are both key phenotypes of BMECs compared to peripheral ECs. By screening the remaining candidate TFs, we aim to identify the roles of these BMEC-enriched TFs in inducing BBB characteristics. Ultimately, we plan to combinatorically express BBB-inducing TFs in hiPSC-EPCs to generate functional BMEC-like cells for drug permeability testing and neurovascular disease modeling.

37 Neutral Sphingomyelinase Regulates Cardiac Mechano-Transduction in Human Engineered Cardiac Tissue

Daniel G. P. Turner[1], Willem J. De Lange[2], Yanlong Zhu[3], Christopher Coe[4], Judith Simcox,[5] Ying Ge[3], Timothy J. Kamp[1], J. Carter Ralphe[2], Alexey V. Glukhov[1]
Departments of Medicine[1], Pediatrics[2], Chemistry[3], Psychology[4], Biochemistry[5], University of Wisconsin-Madison, USA
dgturner@wisc.edu

Cardiovascular disease is the leading cause of death worldwide and is known to be exacerbated by mechanical stress from hypertension. Caveolae are plasma membrane structures that are cardioprotective, buffer mechanical stress, and are downregulated through an unknown mechanism in various cardiovascular diseases. We hypothesized that neutral sphingomyelinases

(nSMase), mechanosensitive enzymes that reside in myocyte caveolae, mechano-activate in response to stretch, generating ceramide and disrupting caveolae and its associated pathways.

Addressing this unknown, we utilized human engineered cardiac tissue (ECT) composed of human-induced pluripotent stem cell-cardiomyocytes and -cardiac fibroblasts, to develop a chronic cyclic stretch protocol.
We evaluated the effects of cyclic stretch using transmission electron microscopy to determine caveolae density, an IonOptix system to determine β-adrenergic response, qPCR to evaluate fibrotic mRNA, LC-MS/MS to estimate ceramide generation, and optical mapping to evaluate the effects of acute nSMase activation in mouse left ventricle.

Our cyclic stretch protocol recapitulates hypertensive cardiac phenotypes such as reduced caveolae expression and a blunted β-adrenergic response. Furthermore, we show that nSMase mechano-activation mediates caveolae downregulation and ceramide generation and that nSMase inhibition prevents stretch-induced blunting of β-adrenergic response. qPCR analysis revealed a limited change in fibrotic mRNA, specifically ACTA2 and COL3A1. Lastly, we showed that stretch- induced conduction slowing in mouse ventricle is nSMase-dependent.

Overall, we utilize advanced stem cell and tissue engineering technologies to produce a novel in vitro stretch protocol that recapitulates changes observed in animal models of cardiac pressure overload and identify nSMase as stretch-induced regulators of cardiac caveolae, contraction, and electrophysiology.

38 Migratory deficits and accelerated maturation of GABAergic neurons in human induced pluripotent stem cell-derived models of *SLC6A1*-Related Developmental and Epileptic Encephalopathy

M. Carmen Varela, Tyler N. Thenstedt, Michael D. Uhler, Jack M. Parent
mcvarela@umich.edu

Myoclonic atonic epilepsy (MAE), a severe developmental and epileptic encephalopathy (DEE), is characterized by seizures, developmental delay and intellectual disability. MAE has been linked to variants in the *SLC6A1* gene encoding the most abundant brain GABA transporter, GAT-1. The GAT-1 transporter regulates the reuptake of GABA at the synapse and, in the cortex, is expressed primarily in interneurons. While loss of function (LOF) of GAT-1 leads to epilepsy and cognitive delays, how GAT-1 knock out and haploinsufficiency affects early human cortical development remains elusive. Here, we use both 2D and 3D human induced pluripotent stem cell (iPSC)-derived models to test the hypothesis that GAT-1 LOF alters interneuron development and subsequent network formation. Compound heterozygous (KO), heterozygous (Het), and isogenic control (WT) *SLC6A1* iPSC lines were generated through concurrent CRISPR/Cas9 gene editing and reprogramming and differentiated into 3D ventral forebrain (VF)-like organoids using our novel self-organizing single rosette spheroid (SOSRS) model, or 2D induced GABAergic neurons (iGNs) using inducible expression of ASCL1 and DLX2. We performed immunolabeling, RT-qPCR, and Western Blot of SOSRS cultured for 2-36 weeks or iGNs for 1-4 weeks to investigate interneuron development and specification. Cortical and VT fusion SOSRS were used to investigate interneuron migration. Both our 2D and 3D WT models displayed appropriate GABAergic and mature neuronal markers and morphology. GAT-1 mRNA expression was decreased 80-95% in KO and 10-70% in HET iGNs and VF SOSRS compared to WT (n=3 lines

each). We observed accelerated GABAergic neuron maturation in KO iGNs and VF SOSRS with earlier increases in potassium-chloride cotransporter 2 (KCC2) and somatostatin expression compared to WT. Additionally, a delayed migration phenotype was observed in KO and Het VF SOSRS, with interneurons exhibiting reduced cell body migration (p=<0.0001) and diminished process extension (p=0.002-0.03). Further investigation into these phenotypes could provide treatment targets for *SLC6A1*-related DEE.

39 DYRK1A Inhibitor Leucettinib-21 Targets Cellular Deficits in Human Trisomy 21 iPSC-derived Neural Cells

Nicole R. West1,2, Mattias Lindberg3, Julien Dairou4, Laurent Meijer3, Anita Bhattacharyya2,5
1Cellular and Molecular Biology Graduate Program, University of Wisconsin-Madison
2Waisman Center, University of Wisconsin-Madison
3Perha Pharmaceuticals, Roscoff, France
4Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, Université Paris Cité, France
5Department of Cell and Regenerative Biology, University of Wisconsin-Madison School of Medicine and Public Health

Dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) is overexpressed in individuals with Down syndrome (DS, trisomy 21). DYRK1A is a potential therapeutic target as it phosphorylates proteins involved in cell cycle regulation, neurogenesis, outgrowth of neurites, synaptic function, and neuronal trafficking. Leucettinib-21 (LCTB-21) is a potent and selective low-molecular weight pharmacological inhibitor of DYRK1A developed to correct cognitive disorders. LCTB-21 has shown promise for improving cognition in rodent models of DS. However, LCTB-21 has not been tested in human cells, and mechanisms by which LCTB-21 improves cognition remain unknown. Using our isogenic pair of control and trisomy 21 (Ts21) induced pluripotent stem cells (iPSCs), we tested the effects of LCTB-21 on DYRK1A activity in human iPSC-derived neural cells to deduce the mechanisms underlying cognitive improvement. Neural progenitor cells (NPCs) and cortical neurons were differentiated from control and Ts21 iPSCs, and we validated that our Ts21 cells have increased DYRK1A protein. LCTB-21 reduces activity of DYRK1A in a dose-dependent manner, however, DYRK1A expression remains stable in NPCs and neurons. LCTB-21 inhibits phosphorylation of Cyclin D1 at T286, a DYRK1A-specific site, in Ts21 and control NPCs. Total Cyclin D1 is increased in Ts21 and control NPCs and neurons, a consequence of its dephosphorylation-induced stability. Cyclin D1 is a regulator of cell cycle progression, however, no significant change in proliferation of Ts21 NPCs was observed within the 24-hour time frame assessed. Ts21 neurons have fewer synapsin-positive synapses relative to controls, and LCTB-21 does not change the number of synapsin-positive synapses in Ts21 neurons. In summary, LCTB-21 targets and inactivates DYRK1A in a dose-dependent manner in human Ts21 iPSC-derived neural cells. LCTB-21 does not increase NPC proliferation, nor does it rescue the number of synapses in Ts21 neurons. Further analysis is required to identify cellular functions affected by reduced DYRK1A activity.

40 Investigating the Programming Event During the Fetal Liver Stage of Hematopoiesis

***Nicole M. Woodhead1*,** Octavia Santis-Larrain1, Sobhika Agarwala1, and Owen J. Tamplin1
1Department of Cell and Regenerative Biology, University of Wisconsin-Madison, USA
nmwoodhead@wisc.edu

Hematopoietic stem and progenitor cells (HSPCs) reside in a microenvironment that regulates their behavior by interactions with niche support cells. During development of the mammalian embryo, fetal liver HSPCs rapidly expand and when transplanted have a greater capacity for reconstituting the blood system compared to more quiescent adult HSPCs. However, the gene

regulatory networks that regulate fetal liver HSPCs are poorly understood. To understand these networks, we are characterizing a viable itga4 mutant zebrafish model with perturbed interaction between HSPCs and the mammalian fetal liver equivalent—the caudal hematopoietic tissue (CHT). We hypothesize that bypass of the CHT niche alters developmental HSPC reprogramming, causing itga4 mutant HSPCs to remain in an immature state. To define the mechanisms of niche reprogramming, we sorted WT and itga4 mutant transgenic Runx:mCherry+ HSPCs by FACS at 5 days post fertilization (dpf) for downstream sequencing and analysis. Bulk RNA-seq analysis detected 335 differentially regulated genes ($q<0.05$; 286 up- and 49 down-regulated transcripts in itga4 mutant compared to WT). Genes upregulated in itga4 mutant HSPCs include klf6a, previously implicated in regulating aged HSPCs, and fosab, a known regulator of differentiation, proliferation, and apoptosis in HSPCs and other cell types. Bulk ATAC-seq data revealed 84,236 accessible regions of chromatin (peaks) unique to WT HSPCs, and 16,875 peaks unique to itga4 mutant HSPCs ($q<0.05$), suggesting that the epigenetic landscape of HSPCs is altered after lodgment in the CHT niche. Motif analysis of peaks unique to itga4 mutant HSPCs unveiled potential regulatory transcription factors of HSPC reprogramming, such as ets1 and AP-1 factors. We will build novel developmental networks of HSPC regulation using multiomic approaches. We will functionally test candidate transcription factors in mammalian HSPCs by overexpression and knockout. Our findings could translate into novel approaches for stem cell expansion and improved stem cell therapy.

41 Investigating the function of DOT1L in cell fate determination

***Xiaoya Zhang*[1,2]**, Coral Wille[2], Rupa Sridharan[2,3]
[1]Department of Genetics, University of Wisconsin-Madison
[2]Wisconsin Institute for Discovery, University of Wisconsin-Madison
[3]Department of Cell and Regenerative Biology, University of Wisconsin–Madison
xzhang2372@wisc.edu

During development transcription factors engage with a conducive chromatin structure to direct differentiation for lineage specification. Chromatin structure is controlled by histone modifications. Pluripotent stem cells (PSCs) have a more open chromatin structure that is conducive to differentiation cues. Surprisingly PSCs have an almost 8 -fold lower level of histone H3 K79 methylation, a modification that is found on actively transcribed genes proportional to their transcriptional activity. H3K79 methylation is mediated by the enzyme Disruptor of Telomeric silencing -1 Like (DOT1L). We have found the inhibiting DOT1L increases histone 3 lysing 9 (H3K9) acetylation (ac) epigenetically, that is without affecting the expression of the enzymes that control histone acetylation levels. This novel contains crosstalk promotes transcription elongation to satisfy the biosynthetic needs of rapidly dividing pluripotent stem cells.