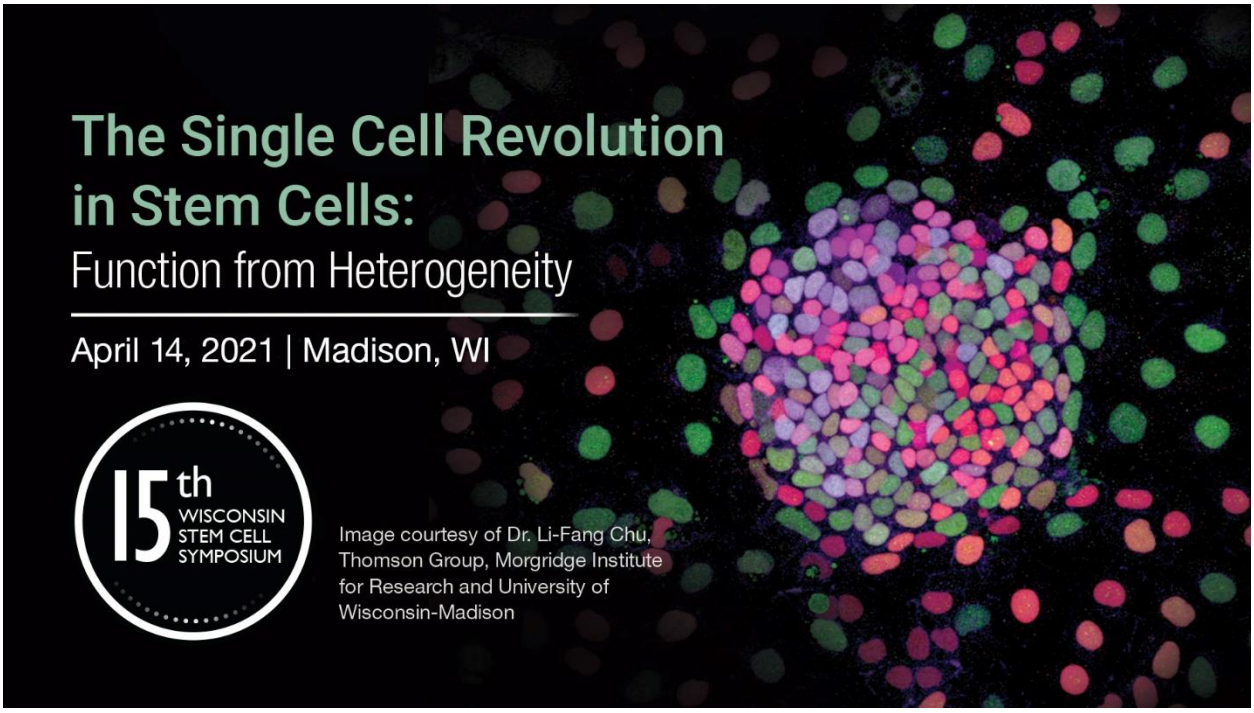

POSTER CONTEST & SESSION

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

CONTEST FINALISTS

(1) Posttranscriptional control of pluripotency by histone demethylases
***Dillingham CM*, Morgan EC, Sridharan R.**
cdillingham1990@gmail.com

Pluripotent stem cells such as embryonic stem cells (ESCs) can self-renew indefinitely and differentiate into any cell type given the right stimulus. This plasticity of ESCs, is linked to a unique chromatin structure. ESCs have a less compacted chromatin structure and low levels of histone modifications associated with gene repression such as methylation at lysine 9 of the histone H3 tail (H3K9me). Among the enzymes that control the levels of H3K9me, we and others have found that the H3K9me1/2 demethylases KDM3A and KDM3b have important roles in the acquisition and maintenance of pluripotency. Double knockout (DKO) of Kdm3a and Kdm3b in ESCs leads to loss of self-renewal, and cell death but the exact cause is unknown. Interestingly, steady state changes in gene expression have not been reporting upon the DKO of these enzymes in ESCs.

To determine the function of these proteins, we performed immunoaffinity purification followed by mass spectrometry and identified associated proteins. Surprisingly, we found that both proteins were associated with RNA processing and export machinery rather than the expected heterochromatin proteins. We orthogonally validated the interaction of KDM3B with splicing target, arginine methyltransferase PRMT5, using proximity ligation assays. We also

found a specific kinase that activates the catalytic activity of the spliecosome was absent in the KDM3 associated proteome. Therefore, we hypothesize that the loss of the KDM3 proteins could affect the expression of alternatively spliced RNA molecules, such as detained introns (DI). DIs are specific internal introns held within transcripts that are held within the nucleus until an external signal induces splicing completion. This mechanism could be used in pluripotent cells to control pluripotency exit during the rapidly changing environment of a developing organism. To interrogate this mechanism, we first generated KDM3B KO ESCs transduced with KDM3A shRNA and performed whole transcriptome RNA sequencing. The results of this analysis will provide insights into the novel post-transcriptional control of pluripotent states by histone demethylases.

(2) A human pluripotent stem cell-derived blood-brain barrier model for studying immune cell interactions

Benjamin D. Gastfriend1, Hideaki Nishihara2, Britta Engelhardt2, Sean P. Palecek1, and Eric V. Shusta1
1 Department of Chemical and Biological Engineering, University of Wisconsin–Madison
2 Theodor Kocher Institute, University of Bern, Switzerland
gastfriend@wisc.edu

The blood-brain barrier (BBB) is formed by brain endothelial cells and serves to tightly regulate molecular and cellular transport between blood and brain parenchyma. BBB dysfunction is a hallmark of multiple sclerosis (MS), in which an abnormally high number of T cells interact with and cross the BBB, subsequently causing demyelination. Our goal was to develop a model of the BBB suitable for the study of such immune cell interactions. Specifically, we were interested in using human pluripotent stem cells (hPSCs), which could be derived from MS patients, to model the BBB. Such a model could eventually be used to ask whether MS patient brain endothelial cells have an intrinsic deficiency contributing to MS pathogenesis. We found that existing hPSC-derived BBB models were unsuitable for study of immune cell interactions as they lacked key adhesion molecules that are known to mediate such interactions. We therefore developed a new model based on stepwise differentiation of hPSCs to endothelial progenitors, naïve endothelial cells, and endothelial cells that begin to acquire moderate barrier properties: extended culture yielded cells with improved expression of the tight junction protein claudin-5 and improved localization of the tight junction protein occludin. Further, after extended culture, permeability to the small molecule tracer sodium fluorescein was similar to existing cell culture models used for immunological studies. Importantly, cells derived via this method expressed key immune cell adhesion molecules (ICAM-1, ICAM-2, VCAM-1, P-selectin, E-selectin) constitutively or in response to inflammatory stimulus. Further, the resulting cells functionally interacted with primary human T cells in an ICAM-1/VCAM-1-dependent manner. In summary, we present a new method to differentiate brain-like endothelial cells from hPSCs that permits investigation of BBB-immune cell interactions in an autologous manner, and should facilitate improved understanding of BBB dysfunction in MS.

(3) Probing spinal cord diversity using hPSC differentiation and single cell sequencing

Nisha Iyer (1,2), Junha Shin (1,3), Stephanie Cuskey (1), Jacky Tian (1,2), Tessa Doersch (1), Noah Nichol (1,2), Sushmita Roy (1, 3), Randolph Ashton (1,2)
(1) Wisconsin Institutes for Discovery, University of Wisconsin—Madison;
(2) Department of Biomedical Engineering, University of Wisconsin—Madison
Ashton Lab, Wisconsin Institutes for Discovery, University of Wisconsin—Madison
(3) Department of Biostatistics and Medical Informatics, University of Wisconsin—Madison
nriyer@wisc.edu

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

(4) Deciphering cell-cell interactions for fine-grained trajectory reconstruction

***Sunnie Grace McCalla1,2*, Junha Shin2, Khagani Eynullazada1,2, Rupa Sridharan2,3, Sushmita Roy2,4**
1. Laboratory 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;
4. Department of Biostatistics and Medical Informatics, University of Wisconsin – Madison
smccalla@wisc.edu

Single-cell RNA sequencing (scRNA-seq) has become an unparalleled technology for examining heterogeneous cell populations. Identification of diverse cell populations and how they relate to each other on a lineage tree is an analytical challenge in many scRNA-seq datasets. Current approaches to define cell populations often use a k-nearest neighbor-based graph of cells, where a cell is connected to the k cells nearest to it determined by gene expression distance values. However, our understanding of how the pairwise nature of k-nearest neighbor graphs affect the ability to capture fine-grained population structure is limited. In particular, pairwise

metrics might not be able to discriminate between direct and indirect dependencies, which can influence the inference of cell-cell interactions and downstream processing. Here we apply probabilistic graph structure learning to scRNA-seq datasets to infer higher order and direct cell-cell interactions. We compare our approach to existing methods of cell type identification and lineage construction on real and simulated datasets from the hematopoietic and cellular reprogramming systems, including a dataset which has a cell barcode-traceable structure to construct cell lineage structure. We assess the methods based on their ability to recapitulate known cell type relationships as well as the quality of cell clusters that are indicative of cell types. We find that our graph-based learning approach is able to accurately infer cell types and identify more biologically meaningful trajectory structures, especially in cell populations that comprise a number of transitioning cells, e.g. in cellular reprogramming. Furthermore, comparison to datasets with available lineage tracing data shows that a graph-based approach is able to better capture lineage structure. Taken together, our graph-based learning framework can recover accurate cell clusters and fine grained cell type relationships that can provide novel insights into the dynamics of a process.

(5) Inhibition of neural stem cell recruitment and differentiation via TGFbeta1 activated Gpnmb on demyelination

Daniel Z. Radecki, Jayshree Samanta
dradecki@wisc.edu

Adult neural stem cells (NSCs) are multi potent stem cells residing within two main regions of the central nervous system (CNS); the forebrain subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ). Following demyelinating insults, resembling the common demyelinating lesions seen in multiple sclerosis (MS), NSC progeny from the SVZ can be diverted from their endogenous olfactory bulb destination to the site of demyelination. There, NSCs can differentiation into oligodendrocyte (OL) lineage cells capable of generating new myelin and repairing the demyelinating insult. Our lab performed an RNAseq analysis on a subset of demyelination responsive SVZ NSCs that express the sonic hedgehog transcription factor Gli1, and identified glycoprotein non-metastatic melanoma b (Gpnmb) as a novel regulator increased during NSC recruitment and oligodendrogenesis. We utilized a Gpnmb-LacZ knockin mouse to generate global Gpnmb-KO mice in which we could simultaneously label Gli1+ NSC via a tamoxifen dependent Gli1-Cre reporter. Mice lacking Gpnmb showed enhanced NSC recruitment and differentiation into OLs following cuprizone mediated demyelination of a major myelin track in the CNS. Our RNAseq also identified the TGFbeta (TGFb) pathway as a potential Gpnmb regulator, and an analysis 5' to the Gpnmb start site identified a Smad3/4 transcription factor binding site, a downstream effector of canonical TGFb1 signaling. In vitro treatment of NSCs with TGFb1 increased Gpnmb expression, while decreasing OL related genes. Interestingly, increased Gpnmb increased TGFb-Receptor 2 (TGFbR2) gene expression, indicating a potential feedforward loop. To test if Gpnmb was blocked by removing TGFbR2, we bred TGFbR2-flox mice to our Gli1-Cre reporter and induced demyelination by stereotaxic injection of the demyelinating compound LPC. This resulted in a significant increase of NSC recruitment and oligodendrogenesis showing that SVZ derived NSC recruitment and repair capability is mediated in part by TGFb1 and Gpnmb.

(6) Integrated Functional Assessments and Top-down Proteomics of Human Induced Pluripotent Stem Cell-derived Cardiomyocytes in Engineered Cardiac Tissues

Jake A. Melbya, ***Kalina Reeseb***, Willem J. de Langec, Jianhua Zhangd, David S. Robertsa, Stanford D. Mitchellb, Trisha Tucholskia, Gina Kimd, Andreas Kyrvasilisb, Sean J. McIlwaine,f, Timothy J. Kampb,d, J. Carter Ralphec*, and Ying Gea,b,g*

aDepartment of Chemistry, University of Wisconsin-Madison, Madison, WI 53706, USA
bDepartment of Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI 53705, USA
cDepartment of Pediatrics, University of Wisconsin-Madison, Madison, WI 53705, USA
dDepartment of Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA
eDepartment of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, WI, 53705, USA
fUW Carbone Cancer Center, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53705, USA
gHuman Proteomics Program, University of Wisconsin-Madison, Madison, WI 53705, USA
kjreese@wisc.edu

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) have enormous potential for disease modeling, cardiotoxicity screening, and drug discovery. 3-dimensional (3D) engineered cardiac tissue (ECT) constructs made from hiPSC-CMs are particularly appealing over 2-dimensional hiPSC-CM monolayers due to their closer representation of the structural and functional complexity of the heart. However, many of the metrics used to assess hiPSC-ECT constructs are performed on separate samples due to incompatibilities between tests. The ability to perform functional and molecular assessments using the same hiPSC-ECT construct is needed for more reliable correlation between observed functional performance and underlying molecular events. Therefore, we developed an integrated method that permits sequential assessment of functional properties and top-down mass spectrometry-based proteomics from the same single hiPSC-ECT construct.1 Our method allowed for quantification of differences in isometric twitch force and post-translational modifications of sarcomere proteins between two groups of hiPSC-ECTs that differed in the duration of time spent in 3D-ECT culture. By using this integrated approach, we discovered a novel correlation between the measured contractile parameters and the phosphorylation levels of alpha-tropomyosin between the two groups of hiPSC-ECTs.

Next we applied our functionally integrated method to CRISPR/Cas9 knockouts (KOs) of cardiac myosin binding protein C (MYBPC3), a thick filament gene often mutated in hypertrophic cardiomyopathy (HCM). hiPSC-ECTs of control and KOs of MYBPC3 have been generated to compare differences in functional assessments and top-down proteomics of sarcomere proteoforms. Our results acquired from the same hiPSC-ECTs indicate that the kinetics, twitch force magnitude, and sarcomere proteoform landscape of the CRISPR-Cas9 KO hiPSC-ECTs were significantly altered compared to the control hiPSC-ECTs. By integrating innovative tissue engineering techniques, functional assessments, and proteomics technologies, we believe this method will be crucial for applications employing hiPSC-ECT for cardiac disease modeling and drug discovery.

(1) Melby, J. A., et al. Functionally Integrated Top-Down Proteomics for Standardized Assessment of Human Induced Pluripotent Stem Cell-Derived Engineered Cardiac Tissues. J. Proteome Res. 2021, 20, 1424–1433. https://doi.org/10.1021/acs.jproteome.0c00830.

(7) Robust identification of cell types and driver genes to characterize the cellular diversity of the human hindbrain and spinal cord

Junha Shin (1), Nisha Iyer (1,2), Sunnie Grace McCalla (1), Stephanie Cuskey (1), Jacky Tian (1,2), Tessa Doersch (1), Noah Nichol (1,2), Randolph Ashton (1,2), Sushmita Roy (1, 3)
(1) Wisconsin Institutes for Discovery, University of Wisconsin—Madison;
(2) Department of Biomedical Engineering, University of Wisconsin—Madison
(3) Department of Biostatistics and Medical Informatics, University of Wisconsin—Madison
junha.shin@wisc.edu

Single-cell RNA-seq (scRNA-seq) enables us to comprehensively profile genome-wide gene expression levels in individual cells. A key problem in the analysis of scRNA-seq datasets is the identification of cell types and the genes that drive the cell type specific expression patterns. While there have been several clustering methods to define cell types, identification of robust cell clusters that are not sensitive to algorithm-specific parameters is a significant challenge. Furthermore, identification of driver genes tends to use pairwise differential expression analysis, which cannot capture genes that could drive subsets of cell types. Here we represent a two-step computational pipeline that first uses consensus clustering with non-negative matrix factorizations to define cell types and then applies multi-task clustering to identify combinatorial patterns of gene expression. We applied our approach to scRNA-seq time course dataset profiling the differentiation of human pluripotent stem cells (hPSCs) into major cell types of the hindbrain and spinal cord spanning 24-216 hours of Hox patterning. Using our clustering approach, we identified 17 groups of cardinal cell clusters that were each further organized into 4-8 hierarchically related subgroups. Our cell clusters are enriched for known neuronal gene markers and recapitulated previously measured mouse in vivo gene expression patterns of major cardinal cell types such as motor, sensory, proprioceptive, and ventral neurons. We applied our multi-task clustering algorithm to identify gene modules for each of the cell subtypes in the cardinal cluster set and identified several genes that exhibit lineage specific patterns of expression. We are validating several of these genes using in situ expression. Taken together, our computational pipeline and novel dataset should be a useful resource for deciphering the cellular complexity of the human hindbrain and spinal cord, and our method should be broadly applicable to scRNA-seq from any complex organ system.

(8) Computational modeling of intercellular signaling during T cell development

Madeline Smerchansky1,2, Krishanu Saha1,2, Melissa Kinney1
Department of Biomedical Engineering1, University of Wisconsin-Madison
Wisconsin Institute for Discovery2, University of Wisconsin-Madison
smerchansky@wisc.edu

Engineered T cell therapies, such as FDA-approved chimeric antigen receptor (CAR) T cells,have shown impressive efficacy in treating CD19+ lymphomas and leukemias. These therapies currently rely on autologous T cells that undergo a lengthy manufacturing process, with the average time to treatment being 23 days1. There is great interest in using induced pluripotentstem cell (iPSC) derived T cells for off-the-shelf immunotherapy2. Current methods for differentiating T cells from pluripotent stem cells rely primarily on undefined mouse cocultures and maturation of the T cells is difficult in vitro3. The unique cues that are important for T cell development in vitro are largely unexplored beyond the reliance on Notch signaling, presented by surrounding stromal cells as delta-like ligand 1 and 4 (DLL1 and DLL4). Quantitative analysis of T cell development in the thymus in vivo could shed light on what signals are

presented by supporting cells to hematopoietic cells during development to inform the manipulation of the in vitro differentiation niche. By using publicly-available single cell RNAseq data from the human thymus4, alongside RNA seq data from an in vitro differentiation method5, a novel model of intercellular communication during T cell development was established using NicheNet, an R tool for analyzing cell-cell communication via RNAseq data6. Ligands that may impact the differences in gene expression between in vitro derived and naïve T cells were determined. This model reveals known ligands, such as DLL4, TNFa, and VCAM-1, as being important during T cell development, as well as many unexplored ligands, such as extracellular matrix proteins laminin and collagen. These results suggest that manipulation of additional signaling pathways in vitro may aid in maturation of iPSC-derived T cells, allowing for efficient generation of off-the-shelf immunotherapies.

References

1. Tyagarajan, S., Spencer, T. & Smith, J. Optimizing CAR-T Cell Manufacturing Processes during Pivotal Clinical Trials. Molecular Therapy - Methods & Clinical Development 16, 136-144 (2020).

2. Iriguchi, S. & Kaneko, S. Toward the development of true "off-the-shelf" synthetic T-cell immunotherapy. Cancer Sci. 110, 16-22, doi:10.1111/cas.13892 (2019).

3. Mohtashami, M. et al. Direct Comparison of Dll1- and Dll4-Mediated Notch Activation Levels Shows Differential Lymphomyeloid Lineage Commitment Outcomes. The Journal of Immunology 185, 867, doi:10.4049/jimmunol.1000782 (2010).

4. Park, J.-E. et al. A cell atlas of human thymic development defines T cell repertoire formation. Science 367, eaay3224, doi:10.1126/science.aay3224 (2020).

5. Montel-Hagen, A. et al. Organoid-Induced Differentiation of Conventional T Cells from Human Pluripotent Stem Cells. Cell Stem Cell 24, 376-389.e378, doi:https://doi.org/10.1016/j.stem.2018.12.011 (2019).

6. Browaeys, R., Saelens, W. & Saeys, Y. NicheNet: modeling intercellular communication by linking ligands to target genes. Nature Methods, doi:10.1038/s41592-019-0667-5 (2019).

GENERAL POSTER SESSION

(9) Modelling the role of APOE4 in blood-brain barrier dysfunctions with isogenic iPSC-derived brain microvascular endothelial cells and pericytes

***Bill Yunfeng Ding*, Richard Daneman, PhD, Sean P Palecek, PhD and Eric V Shusta, PhD**
ding52@wisc.edu

APOE4 is the strongest genetic risk factor for late-onset Alzheimer's Disease, and is known to affect the function of multiple neuronal and glial cell types. Recent research has suggested that blood-brain barrier (BBB) dysfunction is commonly observed among AD patients. However, it remains unclear whether and how APOE4 directly contributes to blood-brain barrier dysfunction. We differentiated isogenic iPSC lines with different APOE isoforms into brain microvascular endothelial cell-like cells (BMECs) and neural crest-derived pericyte-like cells (PCs). Isogenic BMECs with different APOE isoforms were compared for their tight junction integrity, efflux transporter activity and amyloid clearance capabilities. Isogenic PCs with different APOE isoforms were compared for their APOE secretion levels and amyloid uptake capabilities. APOE4, APOE3 and APOE2 BMECs exhibited similar levels of tight junction protein

expression, efflux transporter activities and trans-endothelial electrical resistance. However, we found that the presence of APOE ε4 protein, when compared with APOE ε2 and APOE ε3, led to reduced BMEC clearance of amyloid in a Transwell model. Compared to APOE3 and APOE2 PCs, APOE4 PCs demonstrated similar levels of APOE secretion, but had lower amyloid uptake capabilities. Our findings reveal that although APOE4 did not directly affect BMEC barrier properties in our hPSC-derived model, it plays a potentially important role in amyloid clearance by both BMECs and PCs.

(10) Identifying gene regulatory networks that are causal for cell fate.

Khagani Eynullazada**,** Alireza Fotuhi Siahpirani, Sunnie Grace McCalla, Rupa Sridharan and Sushmita Roy
eynullazada@wisc.edu

One of the fundamental mysteries biology is to understand cell fate specification, which is the process of transitioning from one state (e.g., an embryonic stem cell (ESC) into another (e.g., a skin or a muscle cell). Cell state at any given time is the specified by its expression profile which is maintained by gene regulatory networks (GRNs) defining interactions between regulatory proteins (e.g., transcription factors) and target genes. However, the identity of GRNs that are responsible for cell fate determination are poorly studied. In order to elucidate how GRNs dictates the cell phenotype we used mouse ESC cells as a model to study the role of GRNs in the maintenance of pluripotency. We used expression-based network inference, using the MERLIN tool, on publicly available RNA-seq data from mESCs to infer a global GRN. We prioritized the regulators that are most or establishing ESC state. We tested top 53 regulators inferred by MERLIN, using a high-throughput approach, Perturb-seq, which combines the CRISPR-Cas9 method with scRNA-seq. Perturb-seq allowed us to test our predictions for a large number of regulators in an efficient and cost-effective manner. Nearly all of the gRNAs (52) got efficiently transduced and were represented on average in about 150 cells. Sequencing recovered about 5708 cells, 107 being control (transduced with empty vectors). We integrated the Perturb-seq data with previously published mESC scRNA-seq dataset and found good overlap in the datasets. For several of the regulators, predicted targets exhibited a lower proportion of expressed cells providing support for our computational predictions. Taken together our integrate experimental and computational approach is well-positioned to examine GRNs driving a specific cell fate that we can extend to understand the impact of GRN transitions in cell type specification

(11) Developmental lineage of human pluripotent stem cell-derived cardiac fibroblasts affects their functional phenotype

***Martha E. Floy*[1]**, Sophie E. Givens[2], Jianhua Zhang[1], Timothy J. Kamp[1], Brenda M. Ogle[2], and Sean P. Palecek[1]
[1]University of Wisconsin-Madison and [2]University of Minnesota
mefloy@wisc.edu

Cardiac fibroblasts (CFBs) support heart function by secreting extracellular matrix and paracrine factors, respond to stress associated with injury and disease, and therefore are a promising therapeutic target to treat heart disease. Here, we examine how developmental lineage of human pluripotent stem cell-derived CFBs, epicardial (EpiC-FB) and second heart field (SHF-FB) impacts transcriptional and functional properties. Both EpiC-FBs and SHF-FBs exhibited cardiac fibroblast transcriptional programs. We identified differences between the two fibroblast populations, including in composition of ECM synthesized, secretion of growth and differentiation factors, with EpiC-FBs exhibiting higher stress induced activation potential akin to

myofibroblasts and SHF-FBs demonstrating higher calcification and mineralization potential. These phenotypic differences suggest that EpiC-FBs have utility in modeling fibrotic diseases where activation is important while SHF-FBs are a promising source of cells for regenerative therapies. This work models regional and developmental specificity of CFBs and informs CFB in vitro model selection.

(12) Artificial activation of the Piezo1 mechanosensitive cation channel results in downregulation of efflux transport activity in a stem cell derived model of the blood-brain barrier

Koji Foreman, Eric Shusta, Sean Palecek
klforeman@wisc.edu

The vasculature of the brain is unique within the human body, it screens and regulates the influx of various materials into the brain parenchyma. However, this blood-brain barrier (BBB) also prevents the penetration of pharmaceuticals, creating a roadblock for new neurological drug development. Understanding regulation and behavior of this barrier is pivotal to improving our development of new therapeutics, as well as improving understanding of this unique vascular network. Work from other groups has shown in a mouse model that selective neuronal activation results in a regional decrease in permeability. We hypothesize this reduction in permeability may be through the increased blood flow from neuronal activation and have investigated Piezo1, a unique mechanosensitive protein important to BBB development as a possible contributor to this response. We demonstrate that a Piezo1 agonist reduces efflux activity and increases calcium influx in an in vitro stem cell model of the BBB via a combination of live cell imaging and activity assays. Overall, this suggests that Piezo1 may result in a calcium mediated efflux transport inhibition at the BBB with implications regarding neuronal activity regulating temporarily regulating energy intensive barrier properties.

(13) Human monocytes educated with exosomes from TLR4 primed mesenchymal stem cells treat acute radiation syndrome by promoting hematopoietic recovery

Matthew Forsberg, John Kink, Peiman Hematti, Christian Capitini
mhforsberg@wisc.edu

Developing a cellular therapy that can protect the bone marrow from acute radiation syndrome and stimulate hematopoiesis is a priority for patients exposed to therapeutic or even accidental radiation injury. In this study, exosomes derived from MSCs stimulated with the TLR4 agonist lipopolysaccharide (LPS) were used to alternatively activate human monocytes, termed LPS EEMos, as a potential novel radioprotective cellular therapy. LPS EEMos expressed higher levels of PD-L1 ($p<0.0001$), and lower levels of CD16 ($p<0.01$), CD86 ($p<0.01$), and CD206 ($p<0.0001$) by flow cytometry compared to monocytes educated with exosomes from unstimulated MSCs (EEMos). Using qPCR, increased gene expression in LPS EEMos of IL-10 ($p<0.05$), IDO ($p<0.001$), FGF2 ($p<0.05$), IL-15 ($p<0.05$), and IL-6 ($p<0.0001$) were detected compared to EEMos. Using a xenogeneic radiation injury model, infusion of human LPS EEMos in to NSG mice 4 hours after lethal radiation led to reduced clinical scores and an increased survival at 40 days post-infusion, as compared to infusions of PBS, EEMos, and monocytes alone, all of which led to worse clinical scores and 0% survival with uniform death by 20 days ($p<0.05$). Complete blood cell counts in LPS EEMo recipients showed leukocyte, erythrocyte and platelet counts equivalent to non-irradiated mice, demonstrating complete restoration of hematopoiesis. In vitro co-culture

experiments showed that LPS EEMos were also able to improve the survival of Irradiated human CD34+ haemopoietic stem cells. Due to toxicity concerns, the TLR4 binding, lipid a analogue CRX was used instead of LPS to stimulate MSCs prior to exosome isolation in additional mouse experiments. Results from these experiments showed that the CRX-EEMos provided mice with the same radioprotective effect as LPS-EEMos. Infusion of LPS or CRX EEMos may be a useful strategy to protect the bone marrow from acute radiation syndrome by expression of anti-inflammatory molecules and cytokines that promote hematopoiesis/engraftment.

(14) Lumit™ immunoassay technology for quantifying induced pluripotent stem cells

***Peter Hofsteen*[1]** and Jolanta Vidugiriene[1]
[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711
peter.hofsteen@promega.com

Induced pluripotent stem cells (iPSC) provide an opportunity to explore innovative therapies for degenerative diseases and personalized medicine. Reprogramming iPSCs is a time consuming and inefficient process taking weeks to months to identify positive iPSC-colonies. We sought to identify a non-lytic simplistic add and read assay to detect pluripotent colonies that could be multiplexed and used to streamline the reprogramming process. Lumit™ Technology is a bioluminescent system can be used to detect specific antigens and is built off the NanoBiT® complementation platform. Using a pluripotency cell surface marker, Stage-specific embryonic antigen-4 (SSEA4), we have developed a highly sensitive non-lytic pluripotent detection system that is amenable for high throughput screening and multiplexing. Furthermore, Lumit™ Immunoassays can be adapted to detect any antigen of interest and serves as an alternative for more cumbersome and time-consuming protein detection assays such as ELISA or western blotting.

(15) FXR1 regulation of parvalbumin interneurons in the prefrontal cortex is critical for schizophrenia-like behaviors

Sudharsan Kannan1,2, Minjie Shen1,2, Xinyu Zhao1,2
1 Waisman Center, University of Wisconsin-Madison, Madison, WI 53705, USA
2 Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin Madison, Madison, WI 53705, USA
sudharsan.kannan@wisc.edu

Parvalbumin interneurons (PVIs) are affected in many psychiatric disorders, including schizophrenia (SCZ); however, the molecular mechanism regulating PVIs remains unclear. FXR1(FMR1 autosome homolog 1), a high confident risk gene for SCZ, is indispensable, but its role in the brain is largely unknown. We found that cell type-specific deletion of FXR1 in PVIs of the medial prefrontal cortex (mPFC) reduces PVI excitability, impaired gamma oscillation in mPFC, and SCZ-like behaviors. PVI-specific translational profiling reveals that FXR1 regulates the expression of Cacna1h/Cav3.2, a T-type calcium channel implicated in autism and epilepsy. Inhibition of Cav3.2 in PVIs of mPFC phenocopies, whereas elevation of Cav3.2 in PVIs of mPFC rescues behavioral deficits resulted from FXR1 deficiency. Stimulation of PVIs using a gamma oscillation-enhancing light flicker rescues behavioral abnormalities caused by FXR1 deficiency in PVIs. This work unveils the function of a newly identified SCZ risk gene in SCZ-relevant neurons

and identifies a therapeutic target and a potential non-invasive treatment for psychiatric disorders.

(16) The role of satellite cell derived TRIM28 in mechanical loading-induced and injury-induced myogenesis

Kuan-Hung Lin, Melissa M. Torbey, Jake L. Lemens, Nathaniel D. Steinert, Troy A. Hornberger
School of Veterinary Medicine - Department of Comparative Biosciences, University of Wisconsin-Madison Madison
klin52@wisc.edu

Using a global phosphoproteomics-based approach, we discovered that mechanical loading leads to a highly-robust increase in the phosphorylation of the S473 residue on a protein called TRIM28. Immunohistochemical analysis also revealed that the increase in TRIM28(S473) phosphorylation is largely-mediated by changes that occur within the myonuclei and satellite cells. The alteration in satellite cells was particularly intriguing because satellite cells function as muscle stem cells that can become activated in response to stimuli such as mechanical loading and injury. Once activated, satellite cells can undergo myogenesis, a 3 step process (proliferation, differentiation, fusion) that leads to the addition of new myonuclei into pre-existing myofibers or the generation of new myofibers. Of note, many studies have reported that satellite cells are necessary for the mechanical load-induced growth of pre-existing myofibers, as well as the formation of new myofibers that occur in response to injury. Furthermore, it has been reported that phosphorylation of the S473 residue on TRIM28 can unleash the transcriptional activity of MyoD and Mef2 (two key transcription factors that drive satellite cell-mediated myogenesis). Inspired by these points, we generated satellite cell specific and tamoxifen-inducible TRIM28 knockout mice (KO mice). We then used these mice to discover that TRIM28, within the satellite cells, is required for the normal accretion of myonuclei that occurs in response to mechanical loading as well as the regeneration of myofibers following an injury. Moreover, primary myoblasts obtained from the KO mice revealed a fusion defect during in vitro analyses, and this defect was correlated with a remarkable reduction in fused myofibers and the accumulation of nascent small myofibers in regenerating KO muscles. Taken together, these findings unravel a novel role of satellite cell-derived TRIM28 in mechanical loading-induced and injury-induced myogenesis.

(17) Autofluorescent biomarkers of neural stem cell quiescence

Chris Morrow
cmorrow5@wisc.edu

Neural stem cells (NSCs) create newborn neurons throughout life, yet the rate of production of newborn neurons declines during early-aging and is thought to be limited by the ability of NSCs to exit quiescence. Studies focusing on activated NSCs (aNSCs) and quiescent NSCs (qNSCs) have revealed a great deal about the nature of NSC quiescence exit, however, these studies have been limited by imperfections in the markers used to isolate each population of NSCs. Fluorescence lifetime imaging (FLIM) of endogenous autofluorescent molecules such as the metabolic cofactor NAD(P)H has previously been used as a live-cell, label-free strategy to classify cell activation state by taking advantage of shifts in cellular metabolism which change the optical properties of these autofluorescent molecules. Here we asked whether autofluorescence could be used to discriminate NSC activation state and examined autofluorescent features in aNSCs and qNSCs. Autofluorescent endpoints were significantly shifted between aNSCs and

qNSCs and could be used to accurately classify NSC activation state. More specifically, qNSCs were enriched for an autofluorescent signal localizing to lipid droplets. NSC autofluorescence could enrich for NSC activation state using a cell sorter and track the dynamics of quiescence exit. Finally, autofluorescent punctate signals were detectable in qNSCs in the mouse brain in situ, suggesting the potential for NSC autofluorescence to be used to study NSC quiescence in vivo. Taken together, we describe a live-cell, label-free strategy for determining NSC activation state which expands the toolkit for studying adult neurogenesis.

(18) Establishing the role of essential enhancers in somatic cell reprogramming

Stefan Pietrzak, Sushmita Roy, Rupa Sridharan
spietrzak@wisc.edu

Effective investigation into mechanisms regulating the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) is marred by heterogeneity due to low efficiency (~5%) and variability in kinetics, but can be improved (~40%) through the addition of epigenetic modifying molecules, suggesting that dynamics in regulatory chromatin regions contribute to efficient and successful reprogramming. Reprogramming experiments were performed on mouse embryonic fibroblasts (MEFs) in regular serum-based media and media enriched with a combination of signaling and epigenetic modifiers that synergistically increase reprogramming efficiency. A single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq) was used to investigate chromatin accessibility dynamics in cell populations profiled over the course of the reprogramming timeline. Sequencing data was analyzed using the algorithm ArchR. Single-cell ATAC-seq analysis revealed key insights into the dynamics of chromatin architecture during reprogramming, including a distinction between low- and high-efficiency systems. ArchR clustered the cells based on their accessibility profile rather than their timepoint in the course of reprogramming. A marker gene analysis was performed, which identified accessible gene-associated regions that are differentially opened among the cell population. Intergenic marker peak analysis further identified potential regulatory regions that are differentially accessible. Candidate intergenic peaks of interest will be assayed using CRISPR/Cas9 deletion coupled with reprogramming to determine the role that these intergenic peaks play in successful reprogramming. This analysis of chromatin accessibility reveals differences that may be essential in successful reprogramming dynamics to help further improve its efficiency and expand our knowledge of the process.

(19) Adaptable pulsatile flow generated from stem cell-derived cardiomyocytes using quantitative imaging-based signal transduction

Tongcheng Qian1,*, Daniel A. Gil1,2, Emmanuel Contreras Guzman1, Benjamin D. Gastfriend3, Kelsey E. Tweed1,2, Sean P. Palecek3, Melissa C. Skala1,2,*

1Morgridge Institute for Research, Madison, WI, 53715, USA; 2Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, 53706, USA; 3Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, WI, 53706, USA

*Correspondence should be addressed to T.Q (***tqian5@wisc.edu***) or M.C.S (mcskala@wisc.edu)

Endothelial cells (EC) in vivo are continuously exposed to a mechanical microenvironment from blood flow, and fluidic shear stress plays an important role in EC behavior. New approaches to generate physiologically and pathologically relevant pulsatile flows are needed to understand EC

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

(20) NMR metabolic footprinting of pluripotent stem cell-derived cardiomyocytes for process biomarker discovery

Aaron Simmons
aarondsimmons2@wisc.edu

Heart disease is the leading cause of death in the United States, killing hundreds of thousands of people and costing hundreds of billions of dollars annually. A promising regenerative therapy for reversing damage from heart disease is the implantation of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs); however, several hurdles currently prevent the adoption of said strategy.

One significant impediment to the clinical translation of iPSC-CMs is the time-intensive and low-throughput nature of traditional methods to assess and monitor these cells throughout differentiation. Thus, improved schemes must be developed for the robust, scalable, real-time, continuous monitoring of iPSC-CMs.

In addressing this hurdle, I present metabolic footprinting – analyzing spent cell culture media for metabolite species – as a promising method to assess hPSC-CM phenotype and trajectory throughout their differentiation. Utilizing nuclear magnetic resonance (NMR) spectroscopy, hundreds of unique spectral features can be quantified and correlated to desired phenotypes (in our current studies – hPSC-CM batch purity at Day 16 of differentiation). Such a technique is inherently scalable, non-destructive, and amenable to real-time, closed-loop, continuous monitoring of cultured cells.

Preliminary results demonstrate 1) distinct metabolic signatures among high and low purity hPSC-CM cultures, 2) temporally dynamic metabolic profiles for intermediate cell states through which hPSCs transition en route to hPSC-CMs, and 3) early and continually predictive metabolite profiles which correlate with final hPSC-CM culture purity.

These unique metabolic footprints will allow us to 1) monitor hPSC-CM cultures throughout differentiation, 2) uncover deeper understanding of the underlying biology, 3) develop more robust protocols to differentiate these cells, and 4) inform closed-loop feedback-control systems to further enhance process robustness.

(21) Function of the trinucleotide (CGG) repeats within the FMR1 gene in human neurons

Carissa L. Sirois, Meng Li., Yu Guo, Tomer Korabelnikov, Yina Xing, Bradley Levesque, Anita Bhattacharyya, Xinyu Zhao

clsirois@wisc.edu

The 5' UTR of the FMR1 gene contains trinucleotide CGG repeats, that, when expanded beyond a certain threshold, can lead to one of several disorders depending on the length of the repeats. Repeats that range from 55 to 200 CGGs lead to either Fragile X Tremor and Ataxia Syndrome (FXTAS) or Fragile X Primary Ovarian Insufficiency (FXPOI), while repeats beyond 200 CGGs lead to silencing of the FMR1 gene and cause Fragile X Syndrome (FXS), a neurodevelopmental disorder that causes 2-5% of all autism cases. Recent research has shown that repeats below 55 CGGs are also correlated with certain health conditions, leading to CGG repeat lengths to be further subdivided into low zone (<24 CGG), normal (24-42 CGG), and gray zone (42-54) categories. To investigate the function of these CGG repeats FMR1 in human neurons, we generated two human embryonic stem cell (hESC) lines lacking the CGG repeats in FMR1 (H1ΔCGG, H13ΔCGG) using genome editing and differentiated these cells into neurons. We found that removal of the CGG repeats does not affect FMR1 expression at the mRNA or protein level in either hESCs, neural progenitors, or early post-mitotic neurons. Interestingly, FMR1 mRNA in neurons lacking CGG repeats exhibit altered localization. Our results demonstrate that CGG repeats in FMR1 may have a function in early neural development, which may have important implications for CRISPR-based gene therapy approaches that aim to remove the expanded CGG repeats from the FMR1 gene.

Funding Sources: This study was supported in part by a core grant to the Waisman Center from the National Institute of Child Health and Human Development (U54 HD090256).

(22) Brain aging at single cell resolution in a rapidly aging vertebrate

Teefy, Bryan

teefy@usc.edu

Basic research into the biology of aging is often performed in invertebrate model organisms due to their short lifespan, experimental tractability, and low cost. Thus, our knowledge of vertebrate-specific aspects and drivers of aging biology remain lacking in comparison. Our lab has helped establish the African turquoise killifish (Nothobranchius furzeri) a naturally short-lived vertebrate, as a new model organism to rapidly test basic aging hypotheses in a vertebrate setting. To characterize vertebrate brain aging, we are performing single nucleus RNA-sequencing (snRNA-seq) on the brains of young, middle-aged, and old whole killifish from both sexes and across a short- (GRZ) and long-lived (ZMZ) strain. We plan to quantify changes in cell type and transcriptomic changes that occur during natural aging. Our results should help generate and test hypotheses regarding the drivers of vertebrate brain aging and highlight both strain and sex differences that accompany brain aging.