20 Years of Human Pluripotent Stem Cells:
Current Clinical Trials and Regulatory Framework

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

POSTER CONTEST FINALISTS

(1) Regionally specified human astrocytes exhibit differential molecular signatures and functional properties

Robert A. Bradley, Jack Shireman, Caya McFalls, Jeea Choi, Scott Canfield, Yi Dong, Katie Liu, Brianne Lisota, Jeffery R. Jones, Andrew Petersen, Anita Bhattacharya, Sean Palacek, Eric Shusta, Christina Kendziorski, and Su-Chun Zhang
rabradley@wisc.edu

Astrocytes are known to exhibit different morphologies and functions in different parts of the brain and spinal cord. The origin of this molecular and functional heterogeneity is largely unknown. By generating astrocytes from regionally-specified neural progenitors derived from human pluripotent stem cells, we analyzed the transcription profiles by RNA sequencing and assessed the functional properties and the effects of regional astrocytes on neurons. We found distinct molecular profiles that are associated with each region, including predicted homeodomain transcription factors as well as transcripts which suggest different functional properties. Functional analysis of these regional astrocytes revealed differences in membrane potential and calcium signaling as well as differential effects on neurite outgrowth and blood-brain permeability in co-culture models. These results suggest that differences in regional astrocytes and their functional properties are partly attributed to their developmental origins.

(2) Extensive neurite outgrowth and target specificity from retinal ganglion cells derived from human pluripotent stem cells

Clarisse Fligor, Kirstin B. Langer, Yuan Ren, Valentin M. Sluch, Donald J. Zack, Chi Zhang, Daniel M. Suter, and Jason S. Meyer
cfligor@iupui.edu

Retinal ganglion cells (RGCs) serve as a vital connection between the eye and the brain and as such, the loss of RGCs often leads to blindness. Human pluripotent stem cells (hPSCs) can be differentiated into RGCs, providing an unlimited source of cells for cell replacement strategies. The success of these strategies depend on the ability of RGCs to extend lengthy neurites which integrate with appropriate target tissue. As such, efforts have focused on the development of effective assays to test the ability of hPSC-derived RGCs to extend axons in response to a variety of cues as well as display target specificity. In order to better identify those guidance receptors expressed specifically within hPSC-derived RGCs, the transcriptional profiles of individual cells were analyzed. Results demonstrated that these cells possess receptors that are essential in influencing outgrowth as well as target specificity. Subsequently, the ability of both extrinsic and intrinsic factors to enhance RGC neurite outgrowth was analyzed. Enriched populations of RGCs were isolated and plated to allow for neurite outgrowth, with significant outgrowth observed within the first 24 hours. Finally, to determine target specificity aggregates of hPSC-derived RGCs were co-cultured with explants of mouse lateral geniculate nucleus (LGN), the primary post-synaptic target of RGCs. RGCs displayed target specificity with the longest neurites projecting towards LGN explants. Overall, these results will facilitate the replacement of RGCs following their loss due to disease and degeneration, as extensive axonal outgrowth will be critical for the development of personalized transplant therapies for optic neuropathies.

(3) Microparticles with inorganic nanocoatings improve therapeutic utility of non-viral mRNA delivery

Khalil, A.S. [1], Yu, X. [1], Umhoefer, J.M. [2], Hacker, T.A. [3], Murphy, W.L.[1,4,5,6]

[1]Department of Biomedical Engineering, [2]Department of Biology, [3]Cardiovascular Physiology Core Facility, [4]The Material Science Program, [5]Department of Orthopedics and Rehabilitation, [6]The Stem Cell and Regenerative Medicine Center-University of Wisconsin-Madison, Madison, WI USA

askhalil@wisc.edu

Gene delivery is a widely used approach for regulating protein expression in numerous applications. Classic approaches often utilize either non-viral delivery of plasmid DNA (pDNA) or viral vectors to accomplish this overexpression. However, these methods are typically ill-suited for therapeutic applications due to the low non-viral transfer efficiency of pDNA to non-mitotic cell populations in vivo, as well as the risk of insertional mutagenesis and tumorigenicity of viral vectors, respectively. As an alternative, non-viral delivery of messenger RNA (mRNA) is safe and has high gene transfer efficiency in vivo. However, mRNA delivery is limited by short-lived timeframes of protein overexpression, often on the order of hours. Here we present a biomaterials-based approach, whereby inorganic nanocoatings applied to microparticles (MCMs) provide an efficient and localized non-viral mRNA delivery vehicle for in vivo applications. These MCMs increase the intended biological response of the mRNA delivery via sequestration and stabilization of a cell-secreted overexpressed protein. Specifically, delivery of mRNA encoding for basic fibroblast growth factor (bFGF) via MCMs increased proliferation in primary human fibroblasts two-fold relative to mRNA delivered without MCMs and increased the duration of this biological response. Additionally, MCM-mediated mRNA delivery required a 44-fold lower production of bFGF to match the extent of cell proliferation elicited by treatment with recombinant bFGF protein. Lastly, MCM-mediated delivery afforded local overexpression of proteins in a murine diabetic dermal wound model and resulted in improved rate of wound closure and healing outcomes relative to recombinant bFGF and delivery of mRNA without MCMs. These findings represent a new biomaterials-based mRNA delivery approach, which leverages the innate advantage of high non-viral mRNA transfer efficiency in non-mitotic cell populations and properties of the nanostructured coating to achieve a prolonged and more robust biological response to an overexpressed therapeutic protein.

(4) Blood-derived induced pluripotent stem cells for osteoarthritis modeling

Ming-Song Lee [1,2,#], Lihong Tao[3,#], Brian Walczak[1], Igor Slukvin[3], Wan-Ju Li[1,2]

[1]Department of Orthopedics and Rehabilitation

[2]Biomedical Engineering

[3]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, USA

[#] These authors contributed equally

lee@ortho.wisc.edu

Osteoarthritis (OA) is a disease characterized by irreversible cartilage degradation. However, the mechanisms governing OA progression remain unclear. The primary aim of this study was to establish an OA disease model using induced-pluripotent stem cells (iPSCs). In this study, the peripheral blood mononuclear cells (PBMCs) obtained from both donors with and without OA were epigenetically reprogrammed using the transgene-free episomal vector method and then induced to differentiate into MSCs (iPSC-MSCs) and further into chondrocytes. The results showed that blood-derived iPSCs expressed the typical human ESC markers OCT4, NANOG, SOX2, REX1 and SSEA4. Pluripotency of the iPSCs was confirmed by teratoma formation demonstrating derivatives of all three germ layers. Cells obtained from the iPSC lines exhibited fibroblast-like morphology in culture and expressed CD140a, CD146, CD90, CD105, and CD73 but not CD31 and CD43, suggesting successful derivation of MSCs. After chondrogenic induction, iPSC-MSCs derived from OA donors produced significantly less GAG than those from non-OA

donors. In addition, mRNA levels of markers related to cartilage degradation were significantly upregulated in OA iPSC-MSCs compared to those in non-OA ones. In summary, our findings demonstrate that we have successfully established an OA model using patient iPSCs and the iPSC-based OA model can be used to identify mechanisms of the diseases and potential treatments.

(5) *In vitro* modeling of skeletal muscle pathology using ALS patient-derived induced pluripotent stem cells

***Eileen Lynch*[1]**, Theran Semrad[1], Vincent Belsito[1], Jeremy Jeffrey[1], Masatoshi Suzuki[1,2]
[1]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, USA.
[2]Stem Cell and Regenerative Medicine Center, University of Wisconsin-Madison, Madison, WI, USA.
elynch6@wisc.edu

Amyotrophic lateral sclerosis (ALS) is a late-onset neuromuscular disease with no cure and minimal treatment options. Patients experience a gradual paralysis leading to death from respiratory complications on average only 2-5 years after diagnosis. There is increasing evidence that skeletal muscle is affected early in the disease process, prior even to motor neuron degeneration. Even so, it is largely unknown what pathological processes are occurring in the skeletal muscle of ALS patients. The most common genetic cause of ALS, a hexanucleotide repeat expansion in the *C9ORF72* gene, has yet to be fully characterized in the context of skeletal muscle. In this study, we obtained induced pluripotent stem cells (iPSCs) derived from *C9ORF72* ALS (C9-ALS) patients and used them to create an *in vitro* disease model of C9-ALS skeletal muscle pathology. Using this model, we newly identified *C9ORF72*-specific disease hallmarks that had previously only been characterized in motor neurons. We also found an increased susceptibility to oxidative stress at the progenitor stage as well as increased mitochondrial oxidative stress in mature skeletal myocytes. Further, we performed RNA sequencing and found significant changes in the expression of mitochondrial genes, indicating that mitochondrial dysfunction may be a critical feature of C9-ALS skeletal muscle pathology. Together, these data support the hypothesis that skeletal muscle is independently affected by the ALS disease process. Our study successfully demonstrates the feasibility of ALS patient-derived iPSCs for *in vitro* disease modeling of skeletal muscle pathology, which could lead to the discovery of new therapeutic targets against this devastating disease.

(6) **Engineered anisotropic cardiac tissues**

Brett N. Napiwocki, Di Lang, Ravi Vaidyanathan, Jianhua Zhang, Timothy J. Kamp, Jonathan C. Makielski, Lee L. Eckhardt, Alexey Glukhov and Wendy C. Crone
napiowicki@wisc.edu

Cardiomyocytes derived from human pluripotent stem cells (hPSC-CMs) possess immense therapeutic potential and can serve as innovative pre-clinical platforms for drug development and disease modeling studies; however, methods are lacking to mature hPSC-CMs to levels of adult CMs found in vivo. Building on our previous work of micropatterning CMs on glass substrates, we sought to further improve maturation by combining surface topology with electrical stimulation and a substrate stiffness near that of the healthy myocardium. To enable patterning on substrates with 5-15 kPa stiffness, a trans-print method was employed using a sacrificial polyvinyl alcohol (PVA) film that resulted in the high fidelity pattern transfer of proteins from the film to the soft substrate. Similar to our glass results, we found that the cytoskeletal structure of hPSC-CMs have an adult-like phenotype when micropatterned onto soft substrates. While individual lanes contract independently of one another, the addition of bridges between lanes enabled synchronized contraction over large 2D regions. Unlike monolayers which displayed isotropic electrical impulse propagation, hPSC-CMs grown on micropatterened soft substrates demonstrated anisotropic electrical impulse propagation, as occurs in the native myocardium, with speeds 2x faster in the direction of the lanes compared to

the transverse direction. Moreover, electrical stimulation increased the amount of myofibrils aligned within 10 degrees of the superior angle from 18% in the unpaced monolayer to 28% and 34% when paced at 1Hz and 2Hz, respectively. Similarly, patterned cardiac tissues increased from 61% in the unpaced condition to 64% and 85% in the 1Hz and 2Hz paced conditions. These results demonstrate environmental cues are not mutually exclusive, highlighting the need for multiple signal strategies when engineering cardiac tissues to better mimic the native human heart and create more accurate models for cardiac assays.

(7) Novel platform for transplantation of pluripotent stem cell-derived beta cells

Aida Rodriguez, Sara Dutton Sackett, Dan M Tremmel, Jon S. Odorico
Division of Transplantation, Department of Surgery, University of Wisconsin-Madison School of Medicine and Public Health, Madison, Wisconsin, USA
rodriguezbar@wisc.edu

Human pluripotent stem cell (hPSC)-derived beta cell therapy has been envisioned for diabetic patients as current beta cell replacement therapies are limited by organ shortage, organ quality and immunosuppressive treatment. An ideal beta cell replacement therapy strives towards both generating an abundant supply of functional beta cells and identifying a well-vascularized transplant site. Our hypothesis is that natural human pancreatic extracellular matrix hydrogel (hP-HG) will provide a microenvironment that promotes engraftment and function of hPSC-derived islet-like clusters (ILCs) after transplantation into a prevascularized subcutaneous site (prevasc-SQ). To prevascularize the SQ we implanted an angiocatheter [1] and then removed the catheter 4 weeks later at which time there was CD31+ vessels surrounding the voided space. To first determine if prevascularization and hP-HG are beneficial to the survival of the transplanted cells, mice received islets ± hP-HG in the prevasc-SQ and naïve SQ spaces in immunodeficient mice. Graft histology at 4 weeks post islet transplantation ± P-HG in the prevasc-SQ showed increase of vascularization and islet markers compared to the naïve SQ space. The addition of hP-HG did not appear to further augment the vascularization response in either the naïve or the prevasc-SQ space. We then constructed a composite graft of hPSC-endothelial cells (ECs), mesenchymal stem cells (MSCs), hP-HG and hPSC-ILCs and transplanted them into both sites to determine if this construct can promote vascularization and cell survival without a prevascularization procedure. Immunohistochemistry was performed after graft retrieval at 2 post-transplantation and vascularization was quantified by CD31+ staining. Transplantation of ECs+MSCs+hP-HG+ILCs at 2 weeks in the SQ space demonstrated increased vascularization than the prevasc-SQ site, which suggests that a prevascularization step may not be necessary.
If additional experiments confirm these initial findings, this unique multi-component beta cell transplant strategy could enhance stem cell-derived beta cell engraftment after a minimally invasive SQ transplant.

[1] Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AM. A prevascularized subcutaneous device-less site for islet and cellular transplantation. Nat Biotechnol 2015;33(5):518-523.

(8) Human pancreatic ECM scaffolds for stem cell-derived beta cell culture and transplantation

Daniel M. Tremmel, Sara Dutton Sackett, Austin Feeney, Rachel Maguire, Aida Rodriguez, Sam Mitchell and Jon S. Odorico
tremmel@wisc.edu

Extracellular matrix (ECM) plays a developmental role through structural and biochemical stimulation of cells. Tissue-specific ECM, attained through decellularization, has been proposed in regenerative strategies for organ replacement. Decellularization of animal pancreata has been

reported, but similar methods applied to human pancreas are less effective due to high lipid content. ECM-derived hydrogel can be made from many tissues, but human pancreatic hydrogel has not been reported. Our objective is to produce a biologic scaffold from human pancreatic ECM (hP-ECM) for a variety of uses including stem cell culture and transplantation. Human pancreas was decelled with spin and homogenization techniques, using physical and chemical treatments to isolate hP-ECM. hP-ECM was pepsin digested, neutralized and warmed to 37°C to form hydrogel (hP-HG). hP-ECM and hP-HG were examined for lipid and DNA removal, and retention of ECM proteins and glycosaminoglycans. Cytocompatibility of hP-HG was tested in vitro and immune response was assessed in vivo. Lipid content is significantly reduced following homogenization-decell; lipid removal significantly enhanced gelation of hP-HG. DNA content is significantly reduced in hP-ECM (4.2%) and hP-HG (0.44%) compared to native tissue (100%), while glycosaminoglycan content is moderately retained in hP-ECM (20.8%) and hP-HG (4.0%). Immunogenic proteins, such as HLA Class I and II, are removed during decell. hP-HG is cytocompatible with the beta cell line hINS-1, which grows on hP-HG with equal proficiency as on Col1. When hPSC-derived pancreatic progenitor cells were embedded in hP-HG, the cells retained their Pdx1+ fate, were proliferative (Ki67+) and had low apoptosis levels (Casp3-). When transplanted into humanized mice, hP-HG had minimal cell infiltration whereas antigenic tissue was acutely infiltrated by human T and B cells. Conclusion: We developed a novel protocol for the decellularization of human pancreas and production of hP-ECM and hP-HG scaffolds suitable for cell culture and transplantation applications.

(9) Sporatic Alzheimer's Disease: uncovering new mechanisms and drug targets using patient-derived iPSCs

K.R. Vogel, R. Nichols, K. Kalil, E. W. Dent
Department of Neuroscience, University of Wisconsin, Madison WI 53705
krvogel@wisc.edu

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder and the sixth leading cause of death in the United States. AD is delineated as both familial and sporadic (SAD), with the latter comprising 95% of the cases and has a poorly understood etiology. SAD lacks suitable animal models, however inducible-pluripotent stem cells (iPSCs), reprogrammed stem cells derived from patients' fibroblasts and differentiated into neurons, provide an opportune preclinical model of AD as characterized by GSK3b activation, increased amyloid beta, and tau hyperphosphorylation. Tau plays an important role in morphogenesis, extension of axons, and axonal transport and is intrinsically dysregulated in AD. In the present work, molecular techniques were employed to identify regulators which interact with the neuronal cytoskeleton in patient-derived iPSCs. SAD and age/sex-matched control iPSCs were differentiated into neurons and cultured for 1-3 months. Quantification of protein levels in cell lysates was achieved by Western blot and protein localization was assessed by immunocytochemistry (ICC) followed by confocal microscopy. Neurons expressed measurable levels of neuron-specific β3-tubulin and post-synaptic density protein (PSD-95) by 37 days *in vitro* (DIV 37, post-differentiation). By DIV 75 drebrin positive spine-like protrusions were present on dendrites. SAD neurons extended processes faster than control neurons and had elevated levels of tau expression (4-fold increase, $p<0.03$) by 2 months in culture. Additionally, α-tubulin was elevated 5-fold ($p<0.03$) and modest changes were detected in the microtubule associated protein CLASP1 (2-fold, $p=0.23$), the GTPase Cdc-42 (2.5-fold, $p=0.16$), and the inhibitory synaptic protein gephryin (2.4-fold, $p=0.24$). ICC confirmed neuron-specific upregulation of CLASP1 in neuronal processes (4.6-fold, $p<0.0001$) and revealed that microtubules extend further into the periphery of growth cones. Thus, several proteins integral to MT dynamics (tubulin, tau and CLASP1) are dysregulated in SAD neurons and suggest microtubules may be hyperstabilized, resulting in SAD pathology

(10) Nanostructured microparticles enable sustained release and protein stabilization to reduce the growth factor dosage required for human pluripotent stem cell maintenance

Angela W. Xie[1], Hunter J. Johnson[2], Andrew S. Khalil[1], and William L. Murphy[1,2,3,4]

[1]Biomedical Engineering, University of Wisconsin-Madison; [2]Orthopedics and Rehabilitation, University of Wisconsin-Madison; [3]Materials Science, University of Wisconsin-Madison; [4]Stem Cell and Regenerative Medicine Center, University of Wisconsin-Madison

awxie@wisc.edu

Human pluripotent stem cells (hPSCs) hold vast potential in regenerative medicine, but current methods to expand and differentiate hPSCs at the scale required for cell therapies demand high doses of growth factors that are costly and have limited stability. For example, standard media for expansion of undifferentiated hPSCs (e.g., Essential 8) contain basic fibroblast growth factor (bFGF) at 100 ng/mL concentrations, and the short half-life of bFGF at 37°C necessitates that media be changed daily to maintain pluripotency. We have previously developed nanostructured mineral coatings that can bind, stabilize, and deliver a variety of proteins. Here we hypothesized that stabilization and sustained release of bFGF from mineral-coated microparticles (MCMs) could maintain undifferentiated hPSCs while reducing bFGF usage in long-term culture.

To explore whether MCMs could prolong the biological effects of bFGF in cell culture, we assessed pluripotency marker expression in hPSCs cultured in Essential 8 (E8), E7 (i.e., E8 minus bFGF), and E7+bFGF-loaded MCMs. hPSCs in E7 alone spontaneously differentiated by day 8, while hPSCs cultured with E7+bFGF-loaded MCMs had normal morphology and were >90% Oct4+/Nanog+ after 12 days in direct or indirect culture with MCMs. We used Design of Experiments to optimize bFGF binding to achieve >95% Oct4+/Nanog+ hPSCs while minimizing bFGF usage. Compared to culture in E8, optimized MCMs in direct culture did not require daily bFGF supplementation and thus reduced bFGF usage by >80% while maintaining >95% Oct4+/Nanog+ hPSCs with normal karyotype over 25 passages. hPSCs in direct and indirect culture formats could be transitioned back to E8 with high viability and no apparent MCM carryover within 2 passages, indicating compatibility of this approach with standard hPSC workflows. We posit that this MCM-based strategy for protein stabilization and delivery may overcome limitations in biomanufacturing by reducing the need for costly growth factors in hPSC expansion and differentiation.

GENERAL POSTER SESSION

(11) Exploring altered blood brain barrier endocytosis during bacterial meningitis using iPSC-derived brain endothelial cells

Olivia B. Bee, Brandon J. Kim, Maura A. McDonagh, Eric V. Shusta
University of Wisconsin-Madison, Department of Chemical and Biological Engineering
obee2@wisc.edu

The blood brain barrier (BBB) is comprised of specialized brain microvascular endothelial cells (BMEC) that function to separate the circulation from the central nervous system (CNS). BMECs contain properties, such as complex tight junctions and low endocytosis rates, that together contribute to the BMEC's ability to act as a barrier to pathogens. Certain bacteria, such as Group B Streptococcus (GBS), are able to penetrate the BBB and cause CNS disease such as bacterial meningitis. Bacterial meningitis is a serious infection of the CNS that occurs when bacteria interact with and penetrate the BBB, causing BMECs to respond with a coordinated cellular immune response that contribute to disease progression. Previously, study of the BBB was limited to in vivo models such as rodent and zebrafish models which are limited due to interspecies variation when compared to humans, as well as in vitro immortalized human brain microvascular endothelial cell (BMEC) cell lines, which lose critically defining BBB properties when removed from the brain microenvironment. Recently, a human induced pluripotent stem cell (iPSC)-derived BMEC model has been established that is superior to previously utilized models, such as immortalized and primary cell lines, due in part to the cells ability to retain critical BBB properties, as well as the ability to retain known phenotypes during GBS infection. Using this superior model, we have determined that GBS increases endocytosis rates during infection effectively destroying a defining BBB property. We are currently examining the mechanisms by which GBS is able to disrupt endocytosis rates by exploring specific BBB endocytosis markers. We show how iPSC-derived BBB models are being utilized to examine specific host-pathogen interactions in the context of bacterial meningitis.

(12) P53 Family Members in Cardiac Differentiation of Induced Pluripotent Stem Cells

Emma Brandt, Xing Li, Ph.D., and Timothy J. Nelson, M.D., Ph.D.
Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN.
brandtemma13@gmail.com

Activation of the transcription factor p53 within cancer cells is a well characterized pathway, whereas the effects of p53 activation during development remain largely unexplored. Previous research has indicated that increased levels of p53 protein during key murine developmental stages cause defects in a number of embryonic tissues, including the heart. These findings were confirmed in several different mouse models, but p53 activation in a human system of cardiovascular development is not available. Our lab utilizes induced pluripotent stem cells (iPS cells) derived from patients with congenital heart defects and their parents. These iPS cells undergo an established protocol of cardiac differentiation that allows us to modulate the signaling pathways that would occur during normal human heart development. Characterization of normal p53 levels during cardiogenesis show high levels of p53 protein in iPS cells that decrease upon cardiac differentiation. P53 localization also changes from mainly cytoplasmic in nature in iPS cells to completely nuclear in the cardiac progenitor stage. Pharmacological modulation of p53 protein levels with the Mdm2 inhibitor Nutlin3a for 24 hours during early

(mesoderm to cardiac mesoderm) stages of cardiogenesis resulted in a sizeable loss of cardiomyocytes and reduced expression of cardiac markers days later via increased apoptosis and cell cycle arrest. Interestingly, this increase of p53 levels did not result in apoptosis at later (cardiac progenitor to beating cardiomyocytes) stages of the cardiac differentiation. Further study is being conducted to elucidate the mechanism behind this switch in apoptotic sensitivity in response to elevated p53. The strong nuclear p53 signal in the cardiac progenitor cells suggests p53 could play a previously unidentified role in cardiac commitment and increased levels of p53 could be responsible for loss of cardiomyocytes in a congenital heart defect model.

(13) Coculture of endothelial cells with hPSC-derived cardiac progenitors to enhance cardiomyocyte maturation

Kaitlin K. Dunn, Isabella Reichardt, Sean P. Palecek, Ph.D.
Chemical and Biological Engineering, University of Wisconsin, Madison, WI
kkdunn2@wisc.edu

Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) hold a great potential for cardiac repair. Though we now can generate essentially pure populations of hPSC-derived CMs, these CMs are immature or embryonic-like, causing arrhythmias when implanted into non-human primates. This immaturity is characterized by a difference in cardiac protein expression, electrical and mechanical functionality, and morphology in comparison to adult CMs. Recent research has shown some benefits of coculturing endothelial cells (ECs) with the hPSC-derived CMs to increase the CM maturation. Additional efforts have been made to spontaneously form organized cardiac tissue with the use of cardiac progenitor cells (CPCs) to enhance the tissue function and survival after implantation. Through using hPSC-derived CPCs and hPSC-derived ECs, we demonstrate that the addition of the EC interactions at an earlier point in the CM development has a greater potential for maturation than when coculture is initiated with fully differentiated CMs. Specifically the percent of CMs expressing cTnI, a gene previously not found to be affected by EC coculture, was only significantly increased by the introduction of ECs at the cardiac progenitor stage. The initiation of the coculture at an earlier time in CM development may also stimulate the CMs to mature faster, as shown by the percent of MLC2v+ CMs from the CPC-EC coculture which had no significant difference from the older CMs used in the CM only and CM-EC conditions. This research demonstrates the importance of including interactions with other cell types in the heart at the earliest time possible to create mature hPSC-derived CMs. In the future we will identify the important signals provided by the ECs and determine the differences in CPCs versus CMs that cause them to respond differently to EC interactions.

(14) Cell reprogramming rejuvenates human synovial fluid-derived messenchymal stem cells

Hongli Jiao[1], Brian Walczak[1], Ming-Song Lee[1,2], Wan-Ju Li[1,2]
[1]Department of Orthopedics and Rehabilitation, Laboratory of Musculoskeletal Biology and Regenerative Medicine, University of Wisconsin-Madison, Madison, WI, USA
[2]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, USA
jiao@ortho.wisc.edu

Mesenchymal stem cells (MSCs) hold promise as a therapeutic agent for regenerative medicine; however, their applications are often limited by properties of the cell that are largely dependent on age and health status of the donor. Somatic cell reprogramming capable of altering the epigenetics of a cell may be used to overcome the limitation. In this study, we reprogrammed human synovial fluid-derived mesenchymal stem cells (SF-MSCs) into induced pluripotent stem cells (iPSCs), which were then subsequently differentiated into MSCs to establish iPSC-MSC lines. Activities of iPSC-MSCs and their parental SF-MSCs were compared to determine if reprogramming is able to alter their properties. The results showed that iPSC-MSCs exhibited

the fibroblastic morphology and expressed MSC surface markers. Compared to their parental SF-MSCs, iPSC-MSCs lines increasingly proliferated with reduced cellular senescence in culture and upregulated osteogenesis and chondrogenesis while downregulating adipogenesis. We also found that cell reprogramming led to an increase in telomerase reverse transcriptase (TERT) expression and telomerase activity, which in turn lengthened telomere in iPSCs and derived iPSC-MSCs. Comparing cells at the same population doubling level in culture, the p53/p21 senescence pathway of iPSC-MSCs still remained inactive while that of SF-MSCs was increasingly activated. Our data suggest that iPSC-MSCs are an alternative to MSCs for cell-based therapies and regenerative medicine.

(15) Micropatterned tissue array substrates direct standardized morphogenesis of human pluripotent stem cell derived neural organoids from multiple CNS regions

***Gavin T. Knight*[1,2]** and Randolph S. Ashton[1,2]
[1]Department of Biomedical Engineering, University of Wisconsin-Madison
[2]Wisconsin Institute for Discovery, BioNATES
gknight@wisc.edu

Aggregates of human pluripotent stem cell (hPSC)-derived neural stem cells (NSCs) exhibit cell-intrinsic self-assembly to form bio-mimetic structures with organotypic cell phenotype diversity and microscale tissue cytoarchitecture matching the embryonic central nervous system (CNS). These neural, "organoids" have been used at length to model aspects of human CNS development and neuropathology in settings previously inaccessible for model organisms. However, as neural organoids are typically large aggregates of NSCs, multiple organized regions may form with variable size and shape at indeterminate locations, each representative of disparate regions of the developing brain or spinal cord. The lack of macroscale organization and unpredictable nature of neural organoids complicates tissue maturation and renders tissue reproducibility infeasible. Here we present a custom cell culture platform designed to model the dorsal forebrain and cervical spinal cord regions of the developing neural tube as a modular series of highly standardized neural organoids. Using micropatterned array substrates to restrict NSC aggregate morphology we have identified the optimal physical parameters to facilitate reproducible emergence of neural tissues with a single polarized organizing center or, "neural rosette." Like larger neural organoids, the resulting micropatterned rosette tissues initiate as a single layer of polarized NSCs in spherical/hemi-spherical orientation, but do so in defined areas with consistent dimensions. Further, through modification of the microarray substrate surface chemistry we have demonstrated the ability to alter the cell microenvironment *in situ* to facilitate radial expansion of polarized NSC tissue layers. These results highlight the first step towards a platform for scalable production of standardized neural organoids from multiple CNS regions amenable to real-time analysis, thereby allowing for high-throughput drug screening and disease modelling with human cells in an organotypic context.

(16) Retinal ganglion cell diversity and subtype specification from human pluripotent stem cells

Kirstin B. Langer, Sarah K. Ohlemacher, M. Joseph Phillips, Clarisse M. Fligor, Peng Jiang, David M. Gamm, Jason S. Meyer
klanger@iupui.edu

Retinal ganglion cells (RGCs) are the projection neurons of the retina and transmit visual information to postsynaptic targets in the brain. While this function is shared among nearly all RGCs, this class of cells is remarkably diverse, comprised of multiple subtypes. Previous efforts have identified numerous RGC subtypes in animal models, but less attention has been paid to human RGCs. Thus, efforts of this study examined the diversity of RGCs differentiated from

human pluripotent stem cells (hPSCs) and characterized defined subtypes through the expression of subtype-specific markers. Further investigation of these subtypes was achieved using single cell transcriptomics, confirming the combinatorial expression of molecular markers associated with these subtypes, and also provided insight into novel direction-selective subtype-specific markers. Thus, the results of this study are first to extensively describe the derivation of RGC subtypes from hPSCs and will support

(17) The effect of intermittent normobaric hyperoxia on stem cell mobilization and cytokine expression

***Kent MacLaughlin*[1,2]**, Greg Barton[1,2], Rudolf Braun[1,2], Aleksey Sobakin[1,2], Marlowe Eldridge[1,2]
[1]Department of Pediatrics, [2]John Rankin Laboratory of Pulmonary Medicine
kmaclaughlin@pediatrics.wisc.edu

Introduction/Background
Mechanisms of Hyperbaric Oxygen Therapy (HBOT) putatively include inducing transduction cascades that modulate cytokine expression and mobilize proangiogenic stem/progenitor cells (PSC). Accepted clinical HBOT inhaled oxygen tensions (PIO2) range minimally from 1520 Torr up to 2280 Torr, however, little is known about oxygen therapy below PIO2 760torr. A central dogma in contemporary oxygen therapy research asserts low values of hyperoxia are benign and a useful sham. In this experiment, we measure inflammatory cytokine expression and PSC mobilization at PIO2 320 Torr.

Materials/Methods
Twelve, 10-week-old-Sprague-Dawley rats were randomly divided into two-groups. The treatment group exposed to PIO2 319 torr(41%O2) and the control group exposed to room air. Treatments were administered 5 days/week, 2 hours/day, totaling 20hrs. After sacrifice, monocytes/cells harvested from venous blood were prepared for flow cytometry using antibodies for CD45+, CD34+ and CD133+. Flow cytometry using the BDLSRII/DIVA was analyzed with FlowJo software. Statistics performed using a non-parametric unpaired t-test (Mann-Whitney) with a $p<0.05$ to indicate significance.
Results
Treated animals showed an increase in mobilized CD45+/133+/34- PSC's ($p=0.009$) compared to controls, but no difference in CD45+/133-/34+ ($p=0.99$). TNFα was significantly decreased in treated animals compared to controls ($p=0.004$).
Summary/Conclusions
To our knowledge, this is the first study to demonstrate biologic activity at PIO2 320 Torr. Previous research indicated HBOT mobilizes PSC's with PIO2 1520 Torr. Similar to this finding, our data demonstrates that a much smaller dose PIO2 320 Torr, also mobilizes PSC's and additionally suggests a potential anti-inflammatory effect by reduction in TNFα. Together these findings support the likelihood of biologic activity, consubstantial with HBOT, being activated at much lower dose of hyperoxia than previously postulated. Future research examining oxygen/dose relationship will further elucidate the biological effect of various doses of hyperoxia, and establish differences between concentration and pressure, along with establishing basal active levels.

(18) Bacterial infection alters stem cell-derived blood-brain barrier efflux transporters

***Maura A. McDonagh*[1]**, Brandon J. Kim[1], Olivia B. Bee[1], Kelly S. Doran[2], and Eric V. Shusta[1]
[1]University of Wisconsin-Madison, [2]University of Colorado Anschutz Medical Campus
mcdonagh2@wisc.edu

The blood-brain barrier (BBB) plays a key role in maintaining central nervous system (CNS) homeostasis and is comprised of highly specialized brain microvascular endothelial cells (BMECs). One defining property of the BBB is the presence efflux transporters, such as P-glycoprotein (P-gp), that function to keep toxins and drugs out of the CNS. Study of the BBB *in vitro* has been limited since BMECs, when removed from the brain microenvironment, lose BBB characteristics, including expression of efflux transporters. Animal models, while allowing for study of the BBB within the context of the whole CNS, are inherently limited by the fact that they are non-human. We have developed an induced pluripotent stem cell (iPSC)-derived BMEC differentiation that possesses BBB markers, superior barrier properties, and functional efflux transporters, making it an excellent model for their study. Bacterial meningitis is a life-threatening condition of the CNS that occurs when blood-borne bacteria interact with and penetrate the BBB. Much has been done to characterize bacterial factors and host receptor interactions at the BBB, however, little is known about the role of the efflux transporters during meningitis. Using our BBB model and Group B Streptococcus (GBS) as a model meningeal pathogen, we interrogate efflux transporters during bacterial infection. Our findings suggest that P-gp function is interrupted during infection, leading to a decrease in activity despite an increase in expression. Here for the first time, we demonstrate that an infectious agent such as GBS, can alter efflux transporter function at the BBB. Future studies will seek to examine the subcellular localization of P-gp under control versus infection conditions, aimed at determining the mechanism for the discrepancy between function and expression during infection.

(19) Development of methods to differentiate stem cells into hormone-producing cells: applications in humans, companion animals and agriculture

***Sivan Vadakkadath Meethal*[1]**, Susan DeVries[1], Rastafa Geddes[1], Tina Gonzales[2], Ashley Schwensohn[1], Lisa Krugner-Higby[3] and Craig S. Atwood[1,2]
[1]Division of Product Development, JangoBio, LLC, University Research Park, Madison WI, 53711; [2]Department of Medicine, University of Wisconsin-Madison, WI 53705; and [3]Department of Surgical Sciences, University of Wisconsin-Madison, WI 53705.
sivan.meethal@jango-bio.com

Hypogonadism is a syndrome that results from failure of the gonads (ovaries/testes) to produce sufficient sex steroids, inhibins, anti-Müllerian hormone and numerous other gonadal hormones. All women and men develop hypogonadism; typically at menopause for women, and around 50 years of age for men. Hypogonadism is a major medical challenge, leading to a significant detriment in the quality of life and adversely affecting the function of multiple organ systems. Aside from the well-described symptoms of hypogonadism including reduced libido, infertility, decreased energy, poor concentration and memory, and increased body fat, chronic hypogonadism is strongly associated with several age-related diseases with large treatment markets including Alzheimer's disease, obesity, Type 2 diabetes, and osteoporosis.

To combat hypogonadism, we are developing cell-based biologics to repopulate the gonads to restore gonadal hormone production. Our initial studies focused on the screening of agents that can differentiate stem cells into hormone-producing cells. Rat mesenchymal stem cells (MSCs) grown in the presence of JGO-A1, JGO-A2, JGO-A3, JGO-A4 and JGO-A5 for 7 days increased testosterone production into the media following treatment with N6, 2'-O-dibutyryladenosine

3',5'-cyclic monophosphate sodium (dbcAMP; 1 mM/day; 3 days) by 127%, 32%, 3%, 8% and 20%, compared to controls. JGO-A1 increased estradiol production by 345% and progesterone production by 892% compared to control. A specific combination of agents (JGO-COMBO) was subsequently found to increase testosterone production by 1050% compared to control. Similar in vitro results were obtained with human iPSC, suggesting the modulation of common pathways by these compounds. Preliminary studies of JGO-COMBO-treated MSCs injected into the testes of our middle-aged rat model of hypogonadism indicated the restoration of circulating testosterone concentrations (110% increase) to that of young rats. The development of cell-based biologics that can restore circulating sex hormone concentrations paves the way for their use in the treatment of hypogonadism, as well as a diverse array of applications that includes maintaining or restoring health and well-being in humans, mitigating age-related diseases in humans and companion animals, and in agricultural applications.

(20) Micropatterned substrates for dissecting heterogeneity in reprogramming human somatic cells

***Kaivalya Molugu*[1,2]**, Ty Harkness[1,3], Jared Carlson-Stevermer[1,3], Tiffany Heaster[1,3], Ryan Prestil[1], Stephanie Seymour[1], Gavin Knight[1.3], Randolph Ashton, M[1,3]elissa Skala[1,2,3], Krishanu Saha[1,2,3]
[1]Wisconsin Institute for Discovery, University of Wisconsin-Madison; [2]Department of Biophysics, University of Wisconsin-Madison; [3]Department of Biomedical Engineering, University of Wisconsin-Madison
molugu@wisc.edu

Reprogramming of patient-derived somatic cells to induced pluripotent stem cells (iPSCs) has great promise for disease modeling, drug discovery, and personalized cell therapy. However, this process can take weeks, be stochastic, be inefficient and be partial, resulting in highly heterogeneous populations of cells. Much of the work to identify, evaluate, and enrich for high quality iPSCs has relied on single cell studies which disrupts the cell's microenvironment. However, cell microenvironment has been shown to play an important role in stem cell biology and there has been a lack of understanding of microenvironmental-cell interactions during the middle of reprogramming, precisely when reprogramming cultures are becoming heterogeneous. To address these shortcomings, we developed a micropatterned substrate that allows for dynamic live-cell microscopy of thousands of cell subpopulations undergoing reprogramming. On this substrate, we were able to both watch and physically confine cells into discrete islands during reprogramming while preserving many of the biophysical and biochemical cues within the cells' microenvironment. We are trying to develop regression models based on the nuclear characteristics and the metabolic state of cells to accurately predict their reprogramming status. To this end, we are visually tracking the nuclear characteristics of reprogramming cells within the islands using fluorescence microscopy and we have seen that a combination of eight nuclear characteristics could distinguish partially reprogrammed cells from those that were fully reprogrammed. Additionally, we are tracking the metabolic state of reprogramming cells using a nondestructive technique called Optical Metabolic Imaging(OMI). OMI detects autofluorescence intensity and lifetime of the metabolic co-enzymes NAD(P)H and FAD to probe their relative proportion and binding, thereby quantitating the redox state of the cell. Our preliminary data suggests that OMI is sensitive to the changes in the metabolism of cells during the progression of reprogramming and can be used as a reliable indicator to predict the reprogramming status of cells.

(21) Construction of neurovascular unit models using induced pluripotent stem cells to assess adverse outcomes of chemical exposure

***Eric H. Nguyen*[1,2]**, Debra Fisk[1], Christine M. Sorenson[3], Nader Sheibani[1,2,4]

[1]Human Models for Analysis of Pathways Center, [2]Departments of Ophthalmology and Visual Sciences, [3]Pediatrics, and [4]Biomedical Engineering, University of Wisconsin-Madison, Madison, WI

ehnguyen@wisc.edu

We describe in-vitro neurovascular unit models developed for the detection and characterization of adverse responses to chemicals in high-throughput screening experiments. These models consist of endothelial cells (EC) and astrocytes (AC) derived from human induced pluripotent stem cells (Cellular Dynamics International), as well as pericytes (PC) derived from human brain tissue (ScienCell Labs). The EC, AC, and PC were seeded onto synthetic poly (ethylene glycol) (PEG) hydrogels that were customized with appropriate stiffness and integrin-binding peptide presentation to enable vascular network formation, and the resulting angiogenesis assay recapitulated functionality and cell-cell interactions that are critical for capillary network formation and stability. The cell co-cultures were constructed at multiple ratios to resemble immature neurovascular with low numbers of perivascular supporting cells, as well as mature neurovasculature with elevated numbers of perivascular supporting cells. The models were used to screen a library of 38 chemicals with a range of predicted adverse effects on vascular and neural tissue function. Chemicals that facilitated significant changes in total endothelial network area and area overlap between EC and either PC or AC were categorized as "hits" after fluorescent image analysis. The chemicals were assigned low, medium, high and critical priorities based on the repeatability and magnitude of their effects on EC, PC and AC behavior. The highest-priority chemicals were further characterized through multiple measurements of fluorescent images, including EC network area, EC network protrusions, PC/AC area, and PC/AC overlap with EC networks. Dose response curves generated from these data have demonstrated expected behavior based on the initial chemical screens, as well as AC50 values that were consistent with similar measurements found in the EPA ToxCastTM database. These models promise to identify critical cell types that are affected by chemical exposure, and facilitate the discovery of adverse outcome pathways that compromise the integrity of the neurovasculature.

(22) A label-free method for monitoring metabolic maturation of human pluripotent stem cell derived-cardiomyocytes

***Tongcheng Qian*[1]**, Peter Favreau[1], Kaitlin Dunn[2], Sean Palecek[2], Melissa Skala[1,3]

[1]Morgridge Institute for Research, University of Wisconsin-Madison

[2]Department of Chemical & Biological Engineering, University of Wisconsin-Madison

[3]Department of Biomedical Engineering, University of Wisconsin – Madison

tqian5@wisc.edu

Cardiovascular disease remains the leading cause of death in the world despite advances in treatment. Human pluripotent stem cells (hPSCs) can generate any cell lineage *in vitro*, including cardiomyocytes. hPSC-derived cardiomyocytes have immense potential to impact clinical care and fundamental research for cardiovascular disease. However, hPSC-derived cardiomyocytes exhibit a relatively immature phenotype. New technologies that can non-invasively quantify the maturation state in live hPSC-cardiomyocytes are needed to effectively optimize a mature phenotype. hPSC-derived cardiomyocytes undergo dramatic metabolic changes during maturation. Here, we monitor these metabolic changes that occur in hPSC-derived cardiomyocytes during extended time in culture by multiphoton fluorescence lifetime imaging (FLIM) of the metabolic co-enzymes NAD(P)H and FAD. Changes in hPSC-cardiomyocyte metabolism during maturation were non-invasively quantified at a single-cell level with the

optical metabolic imaging (OMI) index. The OMI index is a linear combination of the optical redox ratio (ratio of the fluorescence intensities of NAD(P)H to FAD), and the mean lifetimes of NAD(P)H and FAD. Our preliminary results indicate that the lifetime of NAD(P)H decreases and the redox ratio increases with maturation over a 60-day time-course in hPSC-derived cardiomyocytes. These label-free imaging technologies could be used to test strategies to optimize the maturation of hPSC-derived cardiomyocytes *in vitro*.

(23) HiBiT: a novel luminescent peptide for rapid CRISPR mediated tagging of endogenous proteins

Thomas Machleidt†, ***Marie K. Schwinn***† , Thomas Machleidt, Kris Zimmerman†, Christopher T. Eggers†, Andrew S. Dixon‡, Robin Hurst†, Mary P. Hall†, Lance P. Encell†, Brock F. Binkowski†, and Keith V. Wood
† Promega Corporation, Madison, Wisconsin 53711, United States
‡ Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112, United States
marie.schwinn@promega.com

One of the key challenges of the post-genomic area is the characterization of protein expression and function in the appropriate physiological context. Dynamic regulation of protein function is achieved through a number of mechanisms including control of protein abundance and post-translational modifications (PTM). Current technologies for the quantitative analysis of endogenous proteins rely heavily on immune-detection which is time consuming and often limited by availability and quality of antibodies. Here we introduce a novel protein tagging method, HiBiT, a multifunctional tag that allows rapid tagging of endogenous proteins via CRISPR/Cas9 mediated gene-editing. The HiBiT tag was developed as part of the NanoLuc Binary Technology (NanoBiT), a complementation system based on NanoLuc luciferase. The 11 amino acid HiBiT peptide and its complementing 18 kDa polypeptide, known as large BiT (LgBiT), spontaneously reconstitute into an active luciferase derived from the NanoLuc enzyme. The detection of HiBiT can be performed in lysate either using a lytic reagent or in living cells by co-expressing the LgBiT and the addition of a live cell substrate. The HiBiT assay system provides over seven logs of linear dynamic range, which makes this technology uniquely for the quantitative analysis of endogenous proteins. To generate the endogenously tagged cell lines, a cloning-free strategy was developed in which cells were transfected with a ribonucleoprotein Cas9:guide RNA complex in the presence of a single-stranded ssODN donor template containing the HiBiT sequence flanked by 50-80 bp homology arms. Within 48 h post-transfection, cell pools can be used for analysis of protein expression. The broadly utility of combining HiBiT technology with CRISPR-mediated gene editing is demonstrated by the generation of multiple cell-based assays that allowed the analysis of protein abundance of multiple proteins along the hypoxia pathway in response to treatment with pathway modulating compounds. In addition the broad utility of HiBiT is further exemplified by the use of HiBit for size analysis using an antibody-free blotting application and BRET (bioluminescence resonance energy transfer) based PTM analysis using PTM specific antibodies. In summary we present here a rapid and simple tagging technology that enables the sensitive detection and quantitation of endogenous proteins with high sensitivity.

(24) Gene and tissue engineering for the treatment of diabetes and its retinal complications

Lalit P. Singh, Ph.D.
Department of Anatomy and Cell Biology**,** Department of Ophthalmology
Wayne State University School of Medicine**,** Detroit, MI 48201, USA
plsingh@med.wayne.edu

Diabetes is a chronic disease in which insulin production is deficient (Type 1) or resistant (Type 2) leading to organ complications including the heart, kidney, retina, and peripheral nerves. About 5-10% of diabetics are Type 1 while ~90% are Type 2 associated with life style changes and obesity. Whether it is Type 1 or Type 2, chronic hyperglycemia prevails and associated oxidative stress and low grade inflammation are considered to play critical roles in diabetes and its complications including diabetic retinopathy (DR). Thioredoxin-Interacting Protein, TXNIP, is strongly induced by diabetes and high glucose in all tissues examined including the pancreatic beta cells and the retina. TXNIP binds to and inhibits the anti-oxidant and thiol reducing capacity of thioredoxins and causes cellular oxidative stress, inflammation and premature cell death. TXNIP is induced strongly by high glucose and its metabolites within minutes and remains elevated as long as hyperglycemia persists. Therefore, the TXNIP gene promoter linked with insulin or a gene of interest may be used to induce gene expression or suppression and in tissue engineering for adult adipose-derived autologous stem cells (ASC) or inducible pluripotent stem cells (iPSC) producing insulin for the treatment of diabetes and its complications such as DR as well as age-related neurodegenerative diseases. For a direct gene therapy to the retina, adeno-associated viral vectors (AAVs) such as AAV2, AAV5, AAV2/8, AAV9 and other modified vectors may be used via an intravitreal or subretinal delivery route.

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(25) Critical aspects of biomanufacturing induced pluripotent stem cells for the treatment of Alzheimer's Disease

Sree Sowmya Tumuluru, UW-Madison Masters in Biotechnology Program
tumuluru2@wisc.edu

Alzheimer's Disease (AD) is a neurodegenerative disease of the cerebral cortex and certain sub-cortical regions characterized by loss of neurons and synapses. The main symptoms of AD patients are cognitive dysfunction and memory loss. Behavioral assessment and cognitive test are the primary diagnosis followed by brain scan to detect neurofibrillary tangles and amyloid plaques in the brain for the AD.

Regenerative stem cell therapy can be a promising approach for rising AD patient survival rate. The success of stem cell therapy depends on the extent of disease phenotyping and the availability of clinical grade stem cells. Induced pluripotent stem cells (iPSCs) are being used extensively for cell-type specific differentiation and have revolutionized the cell therapy approach.

However, it is challenging to manufacture stem cells under cGMP facilities and to follow the regulatory requirements for regenerative therapy products. A robust, reliable and reproducible cGMP compliant iPSC culture system is necessary for the generation of clinical-grade iPSCs. Technical challenges include developing defined, serum-free and feeder-free process for culturing the iPSCs, along with characterization and gene expression profiling of the iPSCs.

This poster entails the details of critical aspects of biomanufacturing the iPSC cells for AD therapy. It also throws light on ethical and regulatory concerns involving stem cell therapy in the US.

(26) Defining reprogramming checkpoints from single cell analysis of induced pluripotency

Khoa Tran[1], ***Zafirah Zaidan[1]***, Stefan Pietrzak[1], Amber Zhou[1], Ali Siapiarahi[1], Gopal Iyer[3], Sushmita Roy[1,4], Rupa Sridharan[1,2]

[1]Epigenetics Theme and -Omics Hub, Wisconsin Institute for Discovery
[2]Dept. of Cell and Regenerative Biology
[3]Dept. of Human Oncology
[4]Dept. of Biomedical Informatics, University of Wisconsin-Madison, WI
nzaidan@wisc.edu

Elucidating the mechanism of reprogramming is confounded by heterogeneity due to the low efficiency and differential kinetics of obtaining induced pluripotent stem cells (iPSCs) from somatic cells. Therefore we profiled the transcriptomes of single cells undergoing accelerated high efficiency reprogramming in the presence of ascorbic acid (AA /Vitamin C), MAP kinase inhibition and Wnt activation (2i), and Dot1l inhibition. Reprogramming MEFs form a single trajectory to an iPSC state with a fraction that exit the trajectory either by failing to downregulate cell migration genes or upregulate embryonic transcription. Surprisingly, MEF-like expression is compatible with progression along the trajectory until the exit points. The functions of each component are separable, with 2i downregulating MEF expression and AA potentiating Dot1l inhibition mediated pluripotency gene expression. Regulatory network analysis identified modules and factors that are associated with specific cell transitions including a novel requirement for translation initiation factor Eif4a1 for the final conversion to an embryonic stem-like state.