TEDCO'S
& ENTREPRENEUR EXPO
STEM CELL SYMPOSIUM

10TH ANNUAL

STEM CELL SYMPOSIUM ABSTRACTS

OCTOBER 30, 2018
THE HOTEL AT THE UNIVERSITY OF MARYLAND

PRESENTED BY:

MSCRF
Maryland Stem Cell Research Fund

ABSTRACT INDEX / POSTER

1

Human Pluripotent Stem Cells with Improved Functionality and Interspecies Chimera Potential

Ludovic Zimmerlin, Tea Soon Park, Rebecca Evans, Justin Thomas, Alice He, Leigh Kulpa, Jeffrey Huo, Riya Kanherkar, and Elias T. Zambidis

Johns Hopkins University-SOM, Baltimore, Maryland

Human naïve pluripotent stem cells (N-hPSC) with improved functionality may have wide impact in human regenerative medicine due to their potential to contribute functional tissues to a developing embryo. For example, stable naïve human embryonic stem cells (N-hESC) may be employed for developing transplantable human organs and adult stem cells in developing animal chimeras, or for generating humanized gene-targeted animal models of disease. Chemical inhibition of GSK3β, ERK and tankyrase signaling (LIF-3i) was recently demonstrated to be sufficient for stable naïve reversion of a broad repertoire of conventional hPSC lines. LIF-3i naïve hPSC (N-hPSC) maintained normal karyotypes, 2-4x fold decreases in global 5-methylcytosine CpG methylation activities, genome-wide CpG demethylation at ESC-specific gene promoters, dominant distal OCT4 enhancer usage, phosphorylated STAT3 signaling, and decreased ERK phosphorylation. LIF-3i increased expressions of naïve-specific human preimplantation epiblast genes (e.g., NANOG, KLF2, NR5A2, DNMT3L, HERVH). Surface marker analysis of N-hPSC vs. their isogenic conventional hPSC counterparts revealed significantly increased expressions of human naïve epiblast-specific markers CD325, CD151, and CD44, and decreased CD24, CD90, and HLA-ABC levels. In contrast to alternative naïve reversion methods that resulted in loss of imprinted genomic regions, LIF-3i N-hPSC were devoid of systematic loss of imprinted CpG patterns or loss of DNA methyltransferase expression (e.g., DNMT1,3A,3B). Methylation array analysis of >1400 known imprinted CpG sites in 12 independent LIF-3i N-hPSC revealed stability of imprints already established in isogenic conventional hPSC. Moreover, LIF-3i naïve reversion resulted in decreased lineage-primed gene expression and improved functional pluripotency, with concomitant improvement in directed differentiation to all three germ layers. For example, LIF-3i N-hPSC generated PAX6+SOX1+Nestin+ neural progenitors, as well as complex retinal organoids more rapidly and efficiently than conventional isogenic hPSC counterparts. To rigorously test functional pluripotency, N-hPSC expressing a GFP-puromycin construct were injected into murine blastocysts and shown to efficiently generate mouse-human chimeric embryos at early stages of murine development via human-specific, quantitative mitochondrial DNA and nuclear antigen expression assays. Later-stage murine embryos showed lower frequencies of human cell incorporation (primarily in murine fetal liver and nervous systems) following in vitro culture selection of chimeras with puromycin. We propose that these LIF-3i N-hPSC with improved functionality and interspecies chimera potency will have great utility in generating humanized disease models, and ultimately in generating patient-specific organs and tissues for regenerative therapies.

2

Nanofiber-Hydrogel Composite-Mediated Neuroprotection After Spinal Cord Injury

Chi Zhang[1-4], Xiaowei Li[1-3], Jerry Yan[1,3,5], Michelle Seu[1,6], Michael Lan[1,3,5], Mingyu Yang[1-3], Brian Cho[1,6], Russ Martin[1-3], Yohshiro Nitobe[7], Kentaro Yamane[7], Aggie Haggerty[7], Ines Lasuncion[7], Megan Marlow[7], Sashank Reddy[6], Da-Ping Quan[4], Martin Oudega[7,8,9], Hai-Quan Mao[1-3,5]

[1]Translational Tissue Engineering Center, [2]Department of Materials Science & Engineering, [3]Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21205, USA, [4]School of Chemistry, Sun Yat-Sen Univeristy, Guangzhou, Guangdong 510275, P. R. China, [5]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21205, USA, [6]Department of Plastic and Reconstructive Surgery, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA, [7]The Miami Project to Cure Paralysis, [8]Department of Neurological Surgery, University of Miami, Miami, FL 33136, USA, [9]Bruce W. Carter Department of Veterans Affairs Medical Center, Miami, FL 33136, USA.

Statement of Purpose: Contusion is the prevalent mechanism of spinal cord injury (SCI) in humans. The efficacy of available therapies for spinal cord contusion is limited, with less than 1% of the patients experiencing complete neurological recovery by the time of hospital discharge. [1] Contusions cause loss of nervous tissue and ultimately the formation of cystic cavities at the injury site, which poses a formidable obstacle for tissue repair and functional recovery. [2] Creating a favorable environment for nervous tissue sparing and regeneration may support improved functional outcomes. We have developed an injectable nanofiber-hydrogel composite (NHC) with interfacial bonding that enables cell infiltration while providing structural integrity for bridging cystic cavities in the injured spinal cord. The purpose of this work was to evaluate the effects and potential mechanisms of NHC on neuroprotection in an adult rat model of spinal cord contusion. Results and Discussion: Gross anatomical evaluation of the contused spinal cords suggested less damage over time at the injury epicenter with the treatment of NHC compared with hydrogels and PBS. Preliminary histological data confirmed decreased tissue loss in contused segments following injection of NHC, especially when compared to other treatments. Furthermore, injection of NHC resulted in the highest densities of blood vessels and axons within the contusion site . The number of inflammatory cells at the contusion site appeared similar among all experimental groups. However, compared to other treatment groups, NHC-treated spinal cord presented the most pro-regenerative M2 macrophage phenotypes (Data not shown). Conclusion: We developed a unique injectable nanofiber-hydrogel composite for supporting repair of the contused spinal cord. Our results suggest that angiogenesis and immunomodulation are possible mechanisms underlying neuroprotection and axon growth in spinal cord lesions.

References:

1. National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. Birmingham, AL: University of Alabama at Birmingham, 2017.

2. Hong, A et al. An Injectable Hydrogel Enhances Tissue Repair after Spinal Cord Injury by Promoting Extracellular Matrix Remodeling. Nature Communications, 2017, 8(1): 1-14.

3

Convertible Natural Killer T-Cells for Cancer Immunotherapy

Susannah C. Shissler, Dominique R. Bollino, Tonya J. Webb
Department of Microbiology and Immunology, University of Maryland School of Medicine and the Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, Maryland 21201

Adoptive cellular transfer (ACT) is an immunotherapeutic strategy used to enhance immune responses in cancer patients. ACT utilizing engineered T cells have the potential to offer long-term protection, through memory, but are directed only towards cancer cells expressing the tumor antigen for which they were designed. This therapy is therefore limited by the possibility of tumor escape – a tumor can downregulate the antigen to which the immunotherapy is directed. An alternate cellular strategy in development utilizes natural killer (NK) cells, which recognize HLA class I molecules. NK cells directly lyse cancer cells, and produce cytokines and chemokines that can activate other components of the immune system. Here, we propose to examine a third type of cellular therapy based on natural killer T (NKT) cells. NKT cells recognize antigen in the context of CD1d, which is expressed in everyone. Moreover, NKT cells can activate both NK cells and classical T cells. Their dual function is ideal for cancer treatment, because NKT cell-based therapy offers the possibility of inducing an innate cytotoxic tumor response, and also activating the adaptive immune system to produce tumor-directed cytotoxic T cells (CTL) with long-lived memory. We have developed a novel method to generate NKT cells from human adult stem cells. We hypothesize that these stem cell-derived "convertible" NKT cells will exert potent antitumor responses. Thus, the goal of this project is to validate our method of generating NKT cells from adult stem cells and further characterize their effector functions. The information gained in the proposed studies will aid in the development of a novel immunotherapy, specifically the generation of universal NKT cells, that can be used for the treatment of cancer.

4

Whole and Phosphoproteome Analysis of Epithelial to Mesenchymal Transition in Human Stem Cell Derived Retinal Pigment Epithelium

Joseph Mertz[1], Srinivas Sripathi[1], Xu Yang[1], Lijun Chen[2], Noriko Esumi[1], Hui Zhang[2], Donald J. Zack[1]

[1]Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Age-related macular degeneration (AMD) is a leading cause of vision impairment among the elderly worldwide and there is evidence that epithelial to mesenchymal transition (EMT) may be involved in AMD pathogenesis in vivo. The advent of embryonic stem (ES) cell-derived RPE cells enables us to investigate EMT-related pathways in human RPE cells in a tightly controlled and systems-based manner. We sought to discern the key protein actors and phosphorylation events in RPE EMT to aid in development of novel therapeutic approaches to modulating this process. Human ES cells were cultured in mTeSR followed by differentiation in media containing 15% KO serum, then matured in RPE medium containing B27. RPE monolayers under five conditions were examined in duplicate: 1) untreated monolayer; 2) enzymatic dissociation and replating for 1hr; 3) enzymatic dissociation + 12hr replating; 4) treatment with TGNF, a combination of transforming growth factor beta (TGFβ) and tumor necrosis factor alpha (TNFα) for 1hr; 5) TGNF treatment for 12hr. Protein was then harvested and prepared for global and phosphoproteome analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). After trypsin digestion, tandem mass tag (TMT) multiplex labelling, and basic reverse-phase LC fractionation, phosphorylated peptides were extracted via Fe3+ immobilized metal affinity chromatography for parallel analysis. All fractions were analyzed on a Q-Exactive MS instrument and peptide sequence matching, phosphosite assignment, and quantification performed using the MaxQuant software package. Pathway analysis on modified proteins and phosphosites was performed using a variety of bioinformatic tools including Ingenuity Pathway Analysis from Qiagen, DAVID pathway enrichment tools from NIH, and the NetworKin kinase substrate analysis suite from the University of Copenhagen. Overall, roughly 8000 proteins and 9500 class I phosphosites were quantified from all ten samples. Interestingly, while protein abundance perturbations overlapped extensively between various groups, among phosphorylation changes the overlap of significant perturbations after 1hr of either dissociation or TGNF was the most extensive of any two combinations of conditions. When normalizing for protein changes the phosphorylation increases shared by these conditions were most intense and widespread in substrates of various MAPKs, while the strongest decreases were observed in substrates of the protein kinase B and C complexes. Both pathways are known to participate in EMT, though the specific phosphorylation events observed here may lead to new therapeutic targets and possibly new means of inducing EMT in vitro.

5

Clinical Evaluation of Longeveron Mesenchymal Stem Cells for Improving Vaccine Response in Aging Frailty

Leng S[1], White O[1], Drouillard A[2], Pena A[2], Peterson K[2], Chisolm C[2], McClain-Moss LR[2], Hare JM[2], Oliva AA[2].

[1]Division of Geriatric Medicine & Gerontology, Johns Hopkins University, Baltimore, MD; [2]Longeveron LLC, Miami, FL

Aging frailty is characterized by the progressive physiologic decline in multiple organ systems, leading to increased vulnerability to disease, comorbidity, and mortality. This includes the age-related decline of the immune system, termed immunosenescence. Immunosenescence results in diminished responsiveness to antigen challenges such as vaccinations, and increases vulnerability to infection and associated complications such as opportunistic infections, increased hospitalization rate, and death. Aging Frailty and immunosenescence appear driven, at least in part, by a chronic pro-inflammatory state, called inflammaging. Therefore, decreasing this pro-inflammatory state could potentially improve immune functioning.In this phase 1/2 clinical trial, we are investigating the potential efficacy of Longeveron-produced allogeneic mesenchymal stem cells (LMSCs) improve immune-response to influenza vaccine in Aging Frailty subjects. LMSCs are a proprietary formulation of mesenchymal stem cells, which are multipotent cells that have powerful anti-inflammatory

properties, and support intrinsic repair and regenerative mechanisms. LMSCs are also immunoprivileged, and thus offer promise as an "off-the-shelf" allogeneic therapeutic.This study entails 3 phases. First was a Run-In Phase to demonstrate provisional safety and tolerability. All subjects of this phase have been treated with LMSCs and vaccinated against influenza. Safety was demonstrated, and the Data Monitoring Committee (DMC) recommended the remainder of the trial proceed without modification. The second phase (Pilot Phase) was designed to evaluate the optimal time-interval between LMSC-treatment and influenza vaccination. All subjects of this Phase have been treated with LMSCs and vaccinated against influenza. Safety was again demonstrated. Preliminary efficacy data also support the hypothesis that LMSCs can improve immune response and immunosenescence in Aging Frailty subjects. The third phase of this study is a Placebo-Controlled, Double-Blinded, Randomized Phase designed to evaluate efficacy. This phase is currently enrolling. We anticipate that the results of this study will demonstrate that LMSC therapy is an efficacious adjuvant therapy that provides significant advantages over influenza vaccine alone. We also anticipate that these results should broadly translate across a spectrum of diseases by restoring back towards normal the functioning of the immune system.

Clinical Evaluation of Longeveron Mesenchymal Stem Cells as An Adjunct Therapy to Surgical Intervention for Hypoplastic Left Heart Syndrome

Kaushal S[1], Mehta H[1], Hibino N[2], Everett AD[2], Gentry T[3], McClain-Moss LR[3], Pena A[3], Peterson K[3], Oliva AA[3], Hare JM[3].

[1]Division of Cardiac Surgery, University of Maryland School of Medicine, 110 S Paca St, 7th Floor, Baltimore, MD; [2]Division of Pediatric Cardiac Surgery and Cardiology, Johns Hopkins, Hospital, Zayed Tower Suite 7107, 1800 Orleans St, Baltimore, MD; [3]Longeveron LLC, 1951 NW 7th Ave, Suite 520, Miami, FL

Hypoplastic left heart syndrome (HLHS) is one of the most complex forms of congenital heart disease, causing roughly 9% of all congenital heart disease. While once uniformly fatal shortly after birth, dramatic improvements in survival had been made through 3-staged surgical palliation that reconstructs the heart to have the right ventricle (RV) support systemic circulation. While these strides have been impressive, the mortality rate nevertheless still approaches 35% during the first year of life, and continues high thereafter. This is largely due to RV failure, and improving RV function in these patients remains a major unmet medical need. We are conducting a Phase 1/2 clinical trial to evaluate the safety and efficacy of allogeneic Longeveron mesenchymal stem cells (LMSCs) as an adjunctive therapy for improving clinical outcomes for HLHS patients. LMSCs are a proprietary formulation of mesenchymal stem cells (MSCs), which are multipotent cells that can support intrinsic repair and rege nerative mechanisms. MSCs can stimulate endogenous stem cell recruitment, proliferation, and differentiation; inhibit apoptosis and fibrosis; and stimulate neovascularization. MSCs are also immunoprivileged, and thus offer promise as an "off-the-shelf" allogeneic therapeutic. The aims of this study are to evaluate the safety and efficacy of intramyocardial injection of LMSCs at the stage II operation for HLHS. We hypothesize that LMSCs will improve the function of the single systemic RV in HLHS patients, and thus improve both short- and long-term clinical measures and outcomes. Our approach is backed by preclinical studies using intracoronary delivery of allogeneic MSCs which led to significant improvement in functional outcomes in the pressure induced right ventricle in a swine model—a model which replicates the physiology present HLHS patients. Phase 1 is an open-label study using 10 HLHS patients. Over 50% of the patients have been treated, with no adverse events (AEs) or serious adverse events (SAEs) attributable to the cell regenerative therapy. Preliminary efficacy data suggests the therapeutic potential of this therapy to improve RV function. Phase 2b is a blinded, randomized study in which 10 subjects will be treated with LMSCs (n=10), or will not receive LMSCs (n=10) during the Stage II operation. This phase will commence after all Phase 1 subjects have been treated, and safety evaluated. This study represents the first allogeneic MSC therapy for HLHS, and we anticipate the results could lead to a paradigm shift in treatment for this highly vulnerable patient population.

Improvement of The Maturation State of Human Induced Pluripotent Stem Cell-Derived 3D Cardiac Microtissues by Defined Chemical Factors

Chen Yu Huang[1], Chin Siang Ong[2], Rebeca Joca[2], Ijala Wilson[2], Deborah DiSilvestre[2], Gordon Tomaselli[2], and Daniel H. Reich[1]

[1]Department of Physics and Astronomy, Johns Hopkins University, Baltimore, MD ; [2]Division of Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD

Recent advances in the understanding and use of pluripotent stem cells have produced major changes in the approach to the diagnosis and treatment of human disease. An obstacle to the use of human pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) for regenerative medicine, disease modeling and drug discovery is their immature state relative to adult myocardium. In this research, 3D cardiac microtissues (CMTs) were generated using hiPSC-CMs to recapitulate the structural, functional and metabolic properties of normal and diseased adult ventricular myocardium. CMTs were treated with ontologically defined biochemical interventions (thyroid hormone, dexamethasone and insulin-like growth factor, TDI) to promote the maturation of hiPSC-CMs. The effects of TDI treatment on both structural and functional (biomechanical, Ca2+ handling and electrophysiology) properties at the tissue level were characterized. In addition, the molecular correlates of maturation of the hiPSC-CMs in the CMTs were studied by gene expression, proteomics studies. Our data demonstrate that TDI treatment improves both structure and function of CMTs. Structurally, the hiPSC-CMs show improved alignment and longer sarcomere length, as demonstrated by immunofluorescence and confocal microscopy. Functionally, the cardiac sarcoplasmic reticulum calcium ATPase-2 (SERCA2) gene, which plays an important role in excitation contraction coupling is increased after treated with TDI. The CMTs' static and dynamic force both increase following TDI treatment, and optical mapping shows that the CMTs are electrically coupled. This, together with the enhanced functional properties that this maturation approach yields has the potential to yield improved model systems that can advance both mechanistic studies and the development of new therapies for the treatment of cardiac diseases.

8

Human Induced Pluripotent Stem Cell-Derived Forebrain Organoids to Assess Neuronal Responses to Radiation-Induced DNA Damage

Debamitra Das[1,3], Mathew Ladra[2], Lawrence Kleinberg[2], Sonia Franco[2] Vasiliki Machairaki[1],[3]* (*co-last authors)*

[1]Department of Neurology, [2]Department of Radiation Oncology and Molecular Radiation Sciences, [3]Institute of Cell Engineering,Johns Hopkins University School of Medicine, Baltimore, MD, USA

Stem cells by virtue of their unique pluripotent capacity can regenerate virtually any cell type and therefore are applied in studies of organ development, disease modeling, drug screening and cell replacement therapy. Induced pluripotent stem cells (iPSCs) under suitable culture conditions in vitro can be differentiated to form an organ like tissue aka 'Organoid' with multiple cell types that closely resembles the true organ in vivo. We hypothesized that human forebrain Organoids may be used as an experimental model to study the mechanisms underlying DNA repair in human neurons at different stages of development, after using radiation-induced DNA damage. To this end, we have designed a protocol for forebrain organoid generation that is reproducible and time saving. These organoids recapitulate key features of human cortical development, including a defined Sub Ventricular Zone (SVZ) containing neural progenitor cells identified by Nestin and SOX2 positive cells that mature to postmitotic cortical neurons as evidenced by TUJ1 and CTIP2 positive cells. Using immunofluorescence (IF) to detect DNA double-strand break (DSB) markers γ-H2AX and 53BP1, we have quantified the kinetics of DSB repair in neural progenitors within the SVZ for up to 24 hours after a single dose (2 Gy) of ionizing radiation (IR). Evaluation of cell survival of both neural progenitors and mature neuronal cells after IR-induced DNA damage using an apoptosis marker CAS3 revealed appearance of apoptotic cells correlated temporally post radiation. Cell cycle analysis using proliferation marker phospho histone3 (PH3) revealed proliferating cells near the ventricular surface of early Organoids that gradually declined post IR. Our data on DNA damage repair in progenitor versus mature neuronal cells indicate a similar timeline by which they repair the DNA damage involving a high incidence of DSB immediately post IR which gets mostly resolved by 18 hours post radiation. On the whole, this study supports the use of 3D Organoid culture technology as a novel preclinical platform to study DNA damage responses in developing and mature neurons.

9

A 3D-Printed Polystyrene Scaffold with Tunable Surface Chemistry to Enhance Mesenchymal Stem Cell Growth and Osteogenic Differentiation

Max J. Lerman[1,2,3],Shin Muramoto[2], Brandon T. Smith[3,4], Anushka Gerald[3,5], James A. Fookes[3,5], Greg Gillen[2], Antonios G. Mikos[3,4], and John P. Fisher[3,5]

[1]Dept. of Materials Science and Engineering, University of Maryland, College Park, MD; [2]Surface and Trace Chemical Analysis Group, Materials Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD; [3]Center for Engineering Complex Tissues, College Park, MD; [4]Dept. of Bioengineering, Rice University, Houston, TX; [5]Fischell Dept. of Bioengineering, University of Maryland, College Park, MD

As cell culture techniques continue to evolve, a balance should be struck between building complex structures that better mimic in vivo conditions and technical ease of use. While polystyrene (PS) has been used as the primary surface for culturing human mesenchymal stem cells (hMSCs), technological advancements in manipulating the surface chemistry could improve targeted protein adhesion and hMSC growth. Additionally, evolution of the chemically complex culture surface from a 2D to a 3D platform is expected to improve intercellular signaling through biomimicry of the native cell environment. Improvement in the design of the 3D scaffold, through investigation into flow effects on hMSC expansion and osteogenic differentiation, could further the development of functional models such as the bone marrow niche for cancer diagnostics and drug discovery. This work seeks to investigate techniques to direct cell fate on bioreactor scaffolds and understand how the native environment can be better replicated in order to build a functional model system. Ultimately, we seek to build a bioreactor system where we can predictably direct long-term hMSC expansion and osteogenic differentiation. Preliminary results show that plasma surface treatment is an effective technique for rapid functionalization of PS surfaces. A glow discharge plasma system operating with 10 SCCM oxygen converted 20.9 ± 1.9 at% of the surface into oxygen containing species, including carbonyl (12.2 ± 4.2 at%) and carboxyl (2.6 ± 1.4 at%) groups, which are seen on surfaces of tissue culture polystyrene (TCPS). Operating with 10 SCCM ammonia converted 11.9 ± 0.4 at% of the surface into nitrogen containing species, primarily as amines (94.7 ± 7.5 at%). The plasma treated surfaces (oxygen, 24.9 ± 2.9°; ammonia 36.0 ± 1.4°) have water contact angles lower than either TCPS (50.0 ± 2.0°) or the native non-treated surfaces (82.9 ± 2.2°). We find that on 2D surfaces, the ammonia treated surfaces have significantly higher DNA content (4.6 ± 0.2x fold change) after 10 days of growth as compared to the oxygen (3.6 ± 0.1x) or TCPS (3.3 ± 0.2x). However, the oxygen treated surfaces have earlier and greater expression of early osteogenic markers (RUNX2 and ALP) and greater calcification (Alizarin Red Staining) over a 3 week time course. This effect is boosted with the addition of osteogenic media. When the 2D surface is converted into 3D scaffolds, we see that the general properties of the functionalized surfaces are conserved. Scaffolds are designed and placed in the treatment chamber with the intent of allowing plasma to penetrate the scaffold interior. After 10 days of static 3D culture, the ammonia (3.7 ± 0.3x) treated 3D scaffolds had statistically greater DNA content than the oxygen (3.0 ± 0.2x) treated scaffolds. In dynamic culture, we see these trends continue up to 7 days, where the ammonia treated scaffolds (2.3 ± 0.3x) supported better growth rather than the oxygen treated scaffolds (0.7 ± 0.2 x) at a flow rate of 3.4 mL/min. Ongoing work includes characterization of osteogenic differentiation on these scaffolds both in static and dynamic culture techniques. Additionally, the influence of surface chemistry and geometry on cytokine production to support hematopoietic stem cell coculture has been the subject of investigation.

10

HMGA1 is Induced by Procarcinogenic Bacteria within the Microbiome to Expand the Colon Stem Cell Pool and Drive Tumorigenesis

Lingling Xian, Li Luo, Lionel Chia, Shaoguang Wu, Cindy Sears, Linda M. S. Resar

Cancer cells undergo chromatin remodeling and epigenetic reprogramming to co-opt stem cell networks and drive tumor progression, although the underlying mechanisms remain poorly understood. High Mobility Group A (HMGA1) chromatin remodeling proteins are architectural transcription factors that bind to DNA at AT-rich sequences where they "open" chromatin and recruit transcriptional complexes to modulate gene expression. The HMGA1 gene is highly expressed during embryogenesis and in adult stem cells, but silenced postnatally in differentiated tissues. HMGA1 is re-expressed in aggressive cancers where high levels portend adverse clinical outcomes. In colorectal cancer, HMGA1 is among the genes most highly overexpressed compared to normal colon epithelium. We previously reported that HMGA1 drives tumor progression in colon cancer by inducing genes involved in an epithelial-mesenchymal transition. We also found that Hmga1 transgenic mice develop aberrant proliferation and polyposis in the small intestine and colon in addition to lymphoid tumors. In small intestinal stem cells, Hmga1 amplifies Wnt signals to enhance self-renewal. Surprisingly, Hmga1 also induces Sox-9 to drive Paneth cell differentiation and "build" a stem cell niche. To determine how HMGA1 functions in colon epithelial homeostasis and carcinogenesis, we examined mouse models with overexpression or deficiency of Hmga1. We discovered that Hmga1 overexpression in colon stem cells also leads to expansion in the colon stem cell pool. Further, both goblet cells and colonic mucous are increased. In dextran sodium sulfate models of inflammatory bowel disease, Hmga1 enhances repair and tissue regeneration, while Hmga1 overexpression promotes aberrant proliferation and polyposis with advancing age in mice. To begin to determine how HMGA1 is induced during carcinogenesis, we examined mice harboring a heterozygous Apc loss-of-function mutation (Min+/-) following colonization with the human symbiotic bacteria, enterotoxigenic Bacterioides fragilis (ETBF). ETBF triggers increased Wnt signaling by inducing E-cadherin cleavage with E-cadherin release, leading to enhanced distal colon tumorigenesis. Importantly, ETBF colonization in humans is linked to both early neoplasia and colon cancer. In preliminary studies, we found that ETBF induces Hmga1 and increases stem cell self-renewal. Inflammatory cytokine genes were also induced. Studies are underway to identify transcriptional networks and epigenetic alterations governed by Hmga1 in this setting. This work not only provides new insights into the role of HMGA1 in colon epithelial homeostasis by maintaining both the stem cell pool and promoting regeneration following injury, but also suggests that HMGA1 can be aberrantly induced by signals from the microbiome to promote tumorigenesis. Our results also highlight the interactions between the microbiome and HMGA1 as a potential therapeutic opportunity in colon carcinogenesis.

11

Characterization of Human IPSC-Derived Extracellular Vesicles to Develop Novel Therapeutic Strategies

Vasiliki Machairaki[1,3], Senquan Liu[2,3] , Kenneth Witwer[4], Linzhao Cheng[2,3]

[1]Department of Neurology, [2]Department of Medicine, [3]Institute for Cell Engineering, [4]Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Human induced Pluripotent Stem Cells (iPSCs) derived from somatic cells of patients provide an unprecedented source of cells for research into future cell therapies. Unlike many adult human stem cells, human iPSCs can be expanded in culture indefinitely and efficiently (10-12-fold every 3 days), while maintaining their developmental potential to generate nearly any cell type. We and others recently developed a completely defined culture system to expand human iPSCs under either adherent or suspension conditions. This system uses the E8 culture medium that is chemically defined with three recombinant proteins plus transferrin, and human recombinant vitronectin as a culture substrate. We analyzed exosomes and/or micro-vesicles (collectively called extracellular vesicles or EVs) made by human iPSCs. We collected iPSC-derived EVs released into the culture conditioned medium and analyzed them following standard methods such as transmission electron microscopy and nanoparticle tracking analysis (NTA) by NanoSight and ZetaView analyzers. We observed a concentration of ~109 particles per ml of conditioned medium, with diameters of 100-150 nm as dominant peaks. The purified EVs by differential ultra-centrifugation contain RNAs, but not genomic DNA. Proteomic and flow cytometric analyses confirmed that they express CD9, CD63 and CD81, as well as many membrane and cytoplasmic proteins of human iPSCs. EVs from human iPSCs stimulated endothelial cell growth in culture. We also investigated if EVs can carry and transport a heterologous protein produced by human iPSCs that stably express the firefly luciferase (fluc) gene as a marker. We confirmed that EVs of the genetically modified iPSCs generated photons once being incubated with a cell-permeable substrate D-luciferin for fluc, indicating the EVs are membrane-enclosed, containing ATP (therefore metabolically active) as well as the fluc heterologous protein. We further investigated the EV uptake and activities in vitro (cell cultures) by monitoring horizontal transfer of the fluc activity into recipient cells. The human iPSCs that are cultured with a highly defined medium (without exogenous EVs that are present in FBS and human biologicals) provide a unique system to analyze unique physical properties as well as their biological composition. We envision that investigating human iPSC-derived EVs will provide novel approaches of using human iPSCs for cellular therapeutics without the whole cells.

This study is supported in part by MSCRF grants.

12

Engineering HPSC-Derived Skeletal Muscle Grafts for The Treatment of Volumetric Muscle Loss

Sarah Somers[1], Jordana Gilbert-Honick[1]*, In Young Choi[1], HoTae Lim[1], Hai-Quan Mao[1], Gabsang Lee[1], Warren L. Grayson[1]*

*[1]Johns Hopkins University, Baltimore, MD, US, * Authors contributed equally to this work*

Tissue engineering has unprecedented potential to create a clinical treatment for volumetric muscle loss (VML) since the standard of care, an autologous muscle flap, is limited by tissue availability and donor site morbidity. However, one of the major factors limiting the use of a tissue engineered strategy to treat VML is the lack of a translatable stem cell source to generate robust, non-immunogenic, tissue-engineered skeletal muscle grafts. To overcome this shortcoming, our group uses human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) derived myoblasts in combination with a novel electrospun fibrin fiber scaffold to engineer skeletal muscle constructs. These cells are jointly referred to as human pluripotent stem cell (hPSC)-derived myoblasts. As previously reported,1 myoblasts can be derived from hPSCs through a 30 day differentiation procedure that includes the use of CHIR99021 over the first 4 days of differentiation followed by 8 days of DAPT. The cells are maintained in serum free N2 media for the remainder of the 30 day differentiation procedure. Following differentiation, cells stain positive for myosin heavy chain (MHC) and myogenin (MYOG) and 30-50% of the differentiated population express dystrophin, Titin, and α-actinin. Currently, our experiments use these hPSC-derived myoblasts, however, in future studies we also plan to explore the effects of cell-sorting on myogenic outcomes in our 3D system, as sorting for a myogenic population has shown promising results following differentiation in a 2D system. Specifically, sorting for the NCAM+/HNK1- population in the hPSC-derived myoblasts has increased the expression of MYOD1 and MYOG ~6 fold over unsorted cells grown in monolayer.1) To create a 3D construct with the hPSC-derived myoblasts, we have developed electrospun fibrin scaffolds with microscale alignment cues and tunable stiffness that in previous studies have enabled robust regeneration of a murine VML defect when seeded with C2C12 myoblasts. 2)The scaffolds contain a porous interior to facilitate cell infiltration and a topographically aligned structure that has caused cellular alignment in a range of cell types including C2C12s, primary human myoblasts, hPSC-derived myoblasts, adipose-derived stem cells, human umbilical vein endothelial cells, and fibroblasts. The scaffolds are also suturable, biodegradable, and offer scale-up capabilities for larger defects. In the current study we have combined our electrospun scaffolds with hPSC-derived myoblasts to develop a translatable tissue engineered skeletal muscle construct.
We have assessed hPSC-derived myoblast growth, morphology, and muscle protein expression on electrospun fibrin scaffolds coated with a range of proteins and seeded at varying densities. hPSC-derived myoblasts proliferated on the scaffolds up to 10 days and expressed desmin with myogenin-positive nuclei, indicative of their pro-myogenic phenotype. The cells also fused to form multinucleated myotubes expressing MF20, a mature muscle marker, and aligned with the scaffold. We have investigated the effect of removing serum from the culture media at different time points and have found that later serum removal resulted in increased proliferation, more myotubes, and a more organized myotube morphology compared to samples with serum removed earlier. In future studies we plan to assess the growth of NCAM+/HNK1- hPSC-derived myoblasts on the fiber as well as asses the ability of the construct to heal a mouse VML defect.

13

Transcriptional Landscape of Human Myogenesis With Multiple Genetic Reporter Embryonic Stem Cell Lines

*HoTae Lim[1], In Young Choi[1], and Gabsang Lee[1, 2, 3, *]*

Stem cell biology with including embryonic stem cells (hESCs) is providing unprecedented opportunity for studying human development. Generating skeletal muscle in vitro with hESCs opens up new avenues for deciphering essential, but poorly understood aspects on transcriptional regulations on skeletal muscle genesis. In this study, our multiple human genetic reporter lines and newly devised myotube isolation method of OCT4::EGFP+ embryonic stem cells, MSGN1::EGFP+ presomite cells, PAX7::EGFP+ putative skeletal muscle stem/precursor cells, MYOG::EGFP+ myoblast cells, and multinucleated myotubes, allow us to depict global gene expression landscape during human skeletal muscle development. We composed a molecular separation of human in vitro myogenesis, and mapped transcriptional changes, named 'virtual myotime' for human skeletal muscle generation. Additionally we defined specialized signature gene expression profiles from each isolated cell population with clustering analysis. Comprehensive transcriptional analysis of hESC-derived myogenic specification process will provide a unique insight including novel transcription factors and signaling pathways. And step-wise RNA-seq profiling and intensive clustering analysis will be informative for understanding gene expression data *in vitro* myogenesis.

14

C-KIT+ Cardiac Progenitor Cells in Closed Chest Swine Model of Myocardial Infarction

David Morales, Gregory Bittle, Rachana Mishra, Sudhish Sharma, Progyaparamita Saha, Muthukumar Gunasekaran, Mir Arfat, Deqiang Li, Sunjay Kaushal

After a myocardial infarction (MI), damaged tissue has minimal healing that occurs in the human heart. The loss of function results in heart failure and increases patients' morbidity and mortality. However, cell-based therapy has shown promising results in myocardial regeneration, especially in myocardial infarction. A recent Phase I clinical trial of autologous (self-donated) cardiac stem cell therapy reported that left ventricular function improved an average of 12% in the year after treatment. However, autologous sources are expensive, time consuming and logistically cumbersome. Allogeneic cardiac stem/progenitor cells (CPCs) generated from neonatal tissue offer an attractive alternative. The Kaushal lab has identified a novel type of CPC that express the cell-surface signaling protein c-kit. These c-kit+ CPCs are able to stimulate the regeneration of cardiac muscle and restore heart function in a rodent model of myocardial ischemia. In a swine myocardial infarction model we hope to demonstrate c-kit+ CPC's can regenerate myocardial tissue after MI in physiology more similar to humans. Furthermore, with a high-dose CPC, low-dose CPC, and a control group this study will demonstrate proof of concept and dosing efficacy parameters for c-kit+ CPC stem cell based regenerative therapy.

15

Tunable Oxygen-Releasing, 3D-Printed Scaffolds Improve *In Vivo* Osteogenesis

***Ashley L. Farris**, Dennis Lambrechts, Nicholas Zhang, S. J. Burris, Alexandra Rindone, Ethan L. Nyberg, Kendall Free, and Warren L. Grayson*

Introduction: The clinical success of tissue engineering approaches is currently limited to acellular, thin, or small scaffolds. One reason for this is the limited oxygen supply when cells are implanted. If a tissue has no vasculature, oxygen is transported through tissue by diffusion alone. To address this, our group previously demonstrated proof-of-concept of a facile method to deliver oxygen to cells using 3D printed scaffolds containing polymeric microparticles, called microtanks, that are hyperbarically loaded with oxygen. However, the translational potential of these microtanks were limited in that they were not composed of biodegradable materials. In this study, we have demonstrated the ability to fabricate biodegradable microtanks, tune oxygen release kinetics from biodegradable, 3D-printed scaffolds by altering the hyperbaric loading pressure and oxygen release kinetics. Furthermore, we have shown that oxygen release from these materials can improve in vivo ectopic bone formation in a murine subcutaneous defect model. Methods: To address the limitations of the commercially available microtanks, we have developed and characterized biodegradable microtanks created using water/oil/water double emulsion. To create the particles, an aqueous solution of polyvinyl alcohol (PVA) is mixed with an organic solution containing poly(lactic-co-glycolic) acid (PLGA). We have performed characterization of these microtanks, examining size, surface morphology, and shell thickness. Particles are then mixed at varying concentrations (10%, 20%, and 30%) with polycaprolactone powder and 3D printed using a custom pneumatic printer. Following printing, the print quality, pore size, strut thickness, surface morphology, and distribution of microtanks through out the scaffold is determined. Scaffolds are loaded with oxygen using a custom-made hyperbaric oxygen chamber pressurized with 100% oxygen at varying pressures for a period of 7 days. To assess the oxygen release from PCL scaffolds without microtank and with varying concentrations of PLGA/PVA microtanks, the oxygen release from hyperbarically-loaded scaffolds and oxygen consumption of adipose-derived stem cells were measured using a Seahorse XF Flux Analyzer for temporal measurements and a Presens fluorescent, oxygen sensitive probe for spatiotemporal oxygen profiles. Release curves were fitted using an exponential decay using a custom Matlab script and cumulative release was determined from the exponential fit. We assessed the impact of oxygen delivery from hyperbarically-loaded microtank scaffolds using a murine subcutaneous defect Finally, we implanted hyperbarically-loaded scaffolds or non-hyperbarically-loaded scaffolds into murine subcutaneous defects (n =8) for 4 weeks and monitored ectopic osteogenesis using computed tomography. Results: We have demonstrated tunable oxygen release kinetics using our novel microtank system. We are able to tune the cumulative loaded oxygen by increasing microtank concentration, with 30% microtank scaffolds containing nearly an order of magnitude higher oxygen than scaffolds without microtanks. Furthermore, we have observed that increasing the loading pressure can increase both the time constant of delivery and the cumulative oxygen loaded in the microtank scaffolds. We have also calculated that 30% microtank-PCL scaffolds can support the oxygen consumption adipose-derived stem cells at 5,000 cells/μL for approximately 35 hours. We also examined the biological implant of microtank-scaffolds on adipose derived stem cell osteogenesis in vivo. When hyperbarically-loaded microtank scaffolds were implanted into murine subcutaneous defects with adipose-derived stem cells, we observed a 63±0.6% increase in bone volume compared to scaffolds that were not hyperbarically loaded. Conclusion: We have demonstrated that microtanks can be fabricated using double emulsion. We have shown that these microtanks can be 3D-printed, hyperbarically loaded, and release oxygen over an extended period of time. Finally, we have demonstrated that oxygen release from microtanks can improve bone formation outcomes. These results highlight both the feasibility and promise of this method to deliver oxygen to stem cells.

16

Treating Timothy Syndrome Through Splice Modulation

***Maria Traficante**[1], Steffi Valdeig[1], Josiah Owoyemi[1], Moradeke Bamgboye[1], Masayuki Yazawa[2], and Ivy E. Dick[1]*

[1]Department of Physiology, University of Maryland; [2]Columbia University Stem Cell Initiative

Timothy Syndrome (TS) is a multisystem disorder, featuring neurological impairment, autism and cardiac action-potential prolongation (long QT) with life-threatening arrhythmias. The life expectancy for these patients is often limited to just a few years of age, due to a lack of adequate treatment options. However, as most TS patients harbor mutations within a mutually exclusive exon, manipulation of splice variation is positioned as a promising and yet unexplored treatment strategy. We therefore aim to expand the treatment options for these patients through splice manipulation. Specifically, we have designed antisense oligonucleotides (AONs) targeted to the deleterious TS exon and evaluated their efficacy in induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs). Application of our AONs to iPSC-CMs derived from a TS patient caused both a decrease in the expression of the deleterious TS exon, as well as a corresponding increase in the non-affected exon. Thus, AON application successfully decreases the burden of TS channels in the heart by replacing them with fully-functional alternatively-spliced channels. Furthermore, AON treatment of the cells corrected the abnormally long action potentials of the TS cells, validating the functional efficacy of this treatment strategy. Overall, splice modulating AONs exhibit a great potential for the treatment of TS patients. Moreover, the approach will serve as a model for developing new therapeutic strategies based on the manipulation of protein splicing, thus enabling a personalized therapeutic strategy for select genetic diseases.

17

Induced Pluripotent Stem Cell-Derived Oligodendrocyte Progenitor Cell Model to Study Neurodegeneration in Progressive Multiple Sclerosis

Itzy Morales[1]*, Vasiliki Marchairaki[1,3], Peter Calabresi[1], and Katie Whartenby[1,2];

[1]Neurology, [2]Oncology, and [3]Institute of Cell Engineering, Johns Hopkins School of Medicine, Baltimore, MD.

Multiple sclerosis (MS) is the most common cause of progressive neurological disability in young adults. It has been characterized as an autoimmune response to myelin, leading to demyelination and disrupting the flow of electrical impulses in the central nervous system. The etiology of MS is unknown but likely involves a combination of genetic, immunological, and environmental factors that influence the pathology, symptomatic presentation, course, and outcome. MS falls predominately into two categories: relapsing-remitting MS (RRMS), and progressive MS (PMS). Advances in disease-modifying therapies have significantly improved the course of RRMS, but PMS remains largely without effective treatments for remyelination and neurodegeneration. Both forms of MS converge on certain pathological features including neuronal cell death and demyelinated axons, but it remains unknown whether the neurons or the oligodendrocytes (OLs) are the main point of defect. Recent studies have shown that oligodendrocyte progenitor cells (OPCs) are present at sites of injury, contradicting the previous belief that adults were unable to remyelinate due to loss of OPCs as a consequence of aging. Yet, remyelination remains inefficient in PMS. MS has been predominately considered an autoimmune process, however, considering the lack of response to immunosuppressants and the presence of OPCs at site of injury, we proposed that the central defect of PMS is not autoimmune but rather a consequence of an inherent inability of OPCs to develop and mature into efficient OLs. The ability to study early pathological processes in MS has been hindered by the inaccessibility of the affected cells, and by a lack of an effective model system to study human OPCs and OLs that closely recapitulates the disease. We have developed a model system based on human induced pluripotent stem cell (iPSC) technology. In this system, peripheral blood mononuclear cells (PBMCs) obtained from a cohort of MS patients are reprogrammed to iPSCs, and further differentiated into OPCs. The OPCs are then exposed to a variety of cytokines known to play a role in the inflammatory environment characteristic of MS, thereby allowing us to identify and monitor early responses, as well as responses to sustained inflammation. In addition to facilitating studies of disease pathogenesis, this new model allows in vitro testing of potential therapies that might prevent OPCs and OLs cell death. Furthermore, this system affords the screening of candidate genes implicated in susceptibility of OL differentiation in MS. Normally, the target cells to be examined in this study, i.e. OPCs would be impracticable to obtain by biopsy in a living person, as the number of participant patients would be insufficient and the yield of cells would be low. In summary, through advances in stem cell technology we have successfully cultivated iPSC-derived OPCs from MS donor blood samples. This enables us to work directly with cells derived from specific individual patients, which is a critical first step into the era of personalized medicine.

18

A Single Cell Transcriptional Atlas of Synovial Joint Development

Qin Bian[1], *Patrick Cahan*[1]

[1]Biomedical Engineering Department, Johns Hopkins University, Baltimore, MD, 21205, USA

Background: Synovial joints are complex anatomical structures, comprising diverse tissue types, including articular cartilage, synovium, fibrous capsule and ligaments, that play a crucial role in skeletal function. Synovial joints development begins with the formation of regions with highly condensed flat mesenchymal cells at the sites of the prospective joint, which is called interzone. The interzone is essential for synovial joint development, if the region is removed through microdissection, the two adjacent bones will fuse and the joint will not be formed. Cells in the central intermediate lamina are progressively lost through apoptosis, leading to the formation of the joint cavity. However, interzone cell fate, roles and modus operandi had long remained obscure. All the above components are derived from an early set of Gdf5-expressing progenitors populating the interzone domain. Lack of GDF-5 in the natural mouse mutant brachypodism, causes widespread joint defects and skeletal growth retardation. Although physiologically and clinically significant there is limited information on the mechanisms and signaling molecules that lead to joint morphogenesis. Despite wide recognition that the interzone region is essential for joint formation, there is limited information of the gene expression environment in which interzone cells emerge and distinguish themselves from adjacent growth plate chondrocytes. A greater understanding of joint formation can provide critical insight on the pathogenesis of joint degeneration and to develop novel reparative strategies to restore long-term joint function following trauma and disease. In this study, we apply Single-cell mRNA-sequencing methods enable unbiased, high-throughput, and high-resolution transcriptomic analysis of individual cells. Methods:Here, we generated single cell RNA sequencing (scRNA seq) data of knee joints from Gdf5-cre::Rosa-EYFP mice at E12.5, E13.5, E14.5, E15.5 and day 5. YFP+ cells were fractionated by fluorescence-activated cell sorting (FACS) and dead cells were excluded by Propidium Iodide. Total 3107, 4786, 1888, 1099 and 4015 cells were profiled respectively by GemCodeTM Single Cell platform (10X Genomics). Data was analyzed by Lovain clustering, differential gene expression and GSEA. We used In situ hybridyzation and FAC-based prospective isolation to verify cell types during synovial joint development. Results: Gdf5 lineage cells collected from E12.5 to day 5 distribute in anlagen, interzone, superficial and deeper zone of articular cartilage, ligament, menisci, synovium and other soft tissue surrounded. E12.5 cells fell into four distinct clusters. One of them is enriched in chondrogenesis related genes such as Col9a, Col2a1 and Sox9, which might localize in outer interzone. This population kept presenting in later stages and contributed to deeper zone of articular cartilage which is verified by ISH. Another cluster highly expressing Col1a1, Col1a2, Osr1 may develop to perichondrium and synovium that surround the joint. The third cluster which is similar to Osr1 enriched cluster may be related to tendon development since two typical markers of tendon progenitors: Scx and Six1 were highly expressed. The cluster enriched in Gdf5 was assumed to contribute to intermediate interzone. Conclusion: Gdf5 lineage cells differentiate to several cell types which contribute to articular cartilage, ligament, synovium and tendon during synovial joint development. The cell fate might be identified by specific gene expressions.

19

Development of A Biphasic MSC Delivery System for The Repair of Osteochondral Defects

George Klarmann, Avi Gnatt, Saya Vijily, Anita Gugel, Yi Arnold and Luis Alvarez; Theradaptive, Inc, Frederick, Md 21704

Theradaptive has developed a platform for a targeted delivery of potent regenerative therapeutics that bring about tissue regeneration in a controlled manner. This technology is based on our discovery of protein sequences that bind to the crystal faces of ceramics extremely tightly. This allows the recombinant production of ceramic binding variants of a wide variety of proteins. Previously, we applied this platform to develop a tetherable variant of BMP2 (tBMP2) that exhibits extremely tight binding of β-TCP ceramic. Thus, we can "paint" the surface of the TCP with tBMP2 to render the inert ceramic into a potent osteoinductive scaffold material. Such targeted delivery potentially allows significant reduction in drug dosage compared to what is used in current state-of-the art products on the market. Under the Maryland Stem Cell Research Foundation, we are advancing the development of our implantable osteochondral repair product called ConForma®. ConForma® is a biphasic osteochondral composite with a thin cartilage-inducing layer overlaying a thick subchondral bone-inducting phase. Our putty design for both phases is made to the consistency of Play-Doh, containing mostly β-TCP. It is customizable, moldable, and conformable, allowing the surgeon to intra-operatively manipulate the shape and size of the implant to render it suitable for use in a wide range of osteochondral repair geometries. It does not rely on any pre-operative customization such as autologous cell expansion or custom machining of hardware component, and the surgeon can apply ConForma® using minimally-invasive arthroscopic surgical techniques. The surgeon can easily load allogenic cells such as MSCs due to the highly porous structure and large surface area. The most important feature of this implant is that novel biologics known for osteoinductive and chondro-inductive are tethered on both phases which can permit cartilage and the subchondral bone repair over timescales exceeding three months. The two objectives of this work are 1. Complete the development of the ConForma® implant system components, and 2. Characterize the performance of ConForma® in cell- based differentiation assays and to develop methods to incorporate MSCCD29+ into the implant while maintaining good viability and engraftment. Since the start of the award in July this year, we have been focusing on the process development of the variants of several growth factors of interest, TFG-β and FGF families in particular. We have developed scalable methods to express our protein in E. coli inclusion bodies. After solubilization and refolding, the proteins were purified using a combination of affinity, ion exchange and hydrophobic chromatography. We have purified up to ~120 mg of active tethered dimeric BMP2 (tBMP2) protein to date. Biological activity of the engineered proteins was confirmed with reporter assays for BMP and TGF-β3, and proliferation assays for FGF, and IGF. Positive binding to TCP was demonstrated for all purified proteins. We are continuing to refine the purification and binding processes, and will approach objective 2 in the coming months.

20

Multi-Lineage Capabilities of Mesenchymal Stem Cells for Engineering Orthopaedic Interfaces

Amelia Hurley-Novatny[1,2], Navein Arumugasaamy[1,2], Megan Kimicata[2,3], John Fisher[1,2]

[1]Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742; [2]Center for Engineering Complex Tissues, University of Maryland, College Park, MD 20742; [3]Department of Materials Science and Engineering, University of Maryland, College Park, MD 20742

Background: Interfaces between orthopaedic tissues, such as bone, tendon, cartilage and muscle, serve to transfer mechanical forces between tissues of differing inherent mechanical properties. Bone and tendon, a stiff tissue and a soft compliant tissue, respectively, are joined by the enthesis. This enthesis has a complex structure characterized by localized and organized extracellular matrix proteins that serve to transfer tensile, compressive, and shear stresses between the two tissues. Following injury of the tendon, surgical intervention typically involves complete resection of the enthesis, resulting in mechanical inferiority and contributing to poor long-term surgical outcomes. Tissue engineering the bone-tendon enthesis provides a potential strategy to improve healing and post-surgical integration. Methods: Bone morphogenic protein-2 (BMP-2), transforming growth factor beta-3 (TGFβ-3), and bone morphogenic protein-12 (BMP-12) were used to induce osteogenic, chondrogenic, and tenogenic differentiation of mesenchymal stem cells (MSC), respectively. MSCs and poly(lactic, co-glycolic acid) microparticles containing either BMP2, TGFβ3, or BMP12, were encapsulated in a gelatin methacrylate (GelMA) hydrogel. These hydrogels were cast in a 3D printed, biocompatible mold. Spatial organization was achieved using a multi-layering technique. Constructs were analyzed for multi-lineage differentiation using PCR to assess cell type-specific gene expression (Runx2 and bone sapiloprotein 1 for osteogenesis, Sox9 and collagen X for fibrochondrogenesis, and scleraxis and tenomodulin for tenogenesis) and staining using IHC for tissue-specific extracellular matrix (ECM) components, including collagen type (I, II, X, and XII), decorin and aggrecan, and hydroxyapetite. Results: Differentiation of cells encapsulated in gels was achieved in monoculture, as assessed by PCR for expression of transcription factors and matrix proteins specific to each lineage. GelMA constructs cast with layers of BMP-2, TGFβ-3, and BMP-12 resulted in regional differences in gene expression consistent with osteogenesis, chondrogenesis, and tenogenesis, respectively. Additionally, constructs had regional differences in deposition of collagen type (I and II), and proteoglycan deposition. Encapsulated cells deposited ECM components specific to the native bone-tendon enthesis, including collagen (X and XII). Conclusions: The ability to differentiate MSCs into multiple lineages in a single construct has previously been a significant challenge, hindering the development of complex and heterotypic tissues. This strategy could potentially be used to aid in treatment of tendon injuries, which typically heal poorly in adults. The overall work demonstrates the potential use of MSCs for development of orthopedic interface tissues such as the bone-tendon enthesis to improve recovery of function following injury.

21

Biliary Atresia Relevant Human iPSCs Recapitulate Key Disease Features in A Dish

Lipeng Tian[1], Zhaohui Ye[2], Kim Kafka[3], Dylan Stewart[4], Robert Anders[5], Kathleen B. Schwarz[3]* and Yoon-Young Jang*[1,6]*

[1]Dept. of Oncology, The Sidney Kimmel Comprehensive Cancer Center; [2]Dept. of Medicine; [3]Department of Pediatrics; [4]Dept. of Surgery; [5]Dept. of Pathology; [6]Institute for Cell Engineering; Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Biliary atresia (BA) is the most common cause of pediatric end-stage liver disease and the most rapidly fibrosing disease in human, but the etiology is poorly understood. There is no effective therapy for BA partly due to lack of human BA models. Towards developing in vitro human models of BA, disease-specific iPSCs from 6 BA patients were generated using non-integrating episomal plasmids. In addition, to determine the functional significance of BA-susceptibility genes identified by genome wide association studies (GWAS) in biliary development, a genome-editing approach was used to create iPSCs with defined mutations in these GWAS BA loci. Using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system, isogenic iPSCs deficient in BA-associated genes (GPC1 and ADD3) were created from healthy iPSCs. Both the BA patient-iPSCs and the knockout (KO) iPSCs were studied for their in vitro biliary differentiation potential. These BA-specific iPSCs demonstrated significantly decreased formation of ductal structures, decreased expression of biliary markers including CK7, EpCAM, SOX9, CK19, AE2 and CFTR and increased fibrosis markers such as alpha SMA, Loxl2 and Collagen1 compared to controls. Both the patient- and the KO- iPSCs also showed increased yes-associated protein (YAP, a marker of bile duct proliferation/fibrosis). Collagen and YAP were reduced by treatment with the anti-fibrogenic drug pentoxifylline. In summary, these BA-specific human iPSCs showed deficiency in biliary differentiation along with increased fibrosis, the two key disease features of BA. These BA-relevant iPSCs can provide new human BA models for understanding the molecular basis of abnormal biliary development and fibrogenesis, and opportunities to identify drugs that have therapeutic effects on BA.

22

Maintaining Human Pluripotent Stem Cells with Optical Stimulation of the FGF Signaling Pathway

In Young Choi[1], HoTae Lim[1], and Gabsang Lee[1, 2, 3, *]*

[1]The Institute for Cell Engineering, [2]Dept. of Neurology and Neuroscience, [3]The Solomon H. Synder Department Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Stem cell fate is largely determined by a cell-signaling network and can be controlled by the supplementation of exogenous recombinant proteins. However, this may cause heterogeneous and unsynchronized signaling due to the uneven distribution of recombinant proteins and poor thermostabilty. Such issues are closely associated with the spontaneous differentiation of human pluripotent stem cells (hPSCs), which may hamper future stem cell applications. Here, we introduce a novel hPSC culture system to employ optical stimulation of the fibroblast growth factor (FGF) signaling pathway, without the daily supplementation of recombinant FGF2 protein (a key molecule for their stemness). With CRISPR/Cas9-based genetic incorporation of the large light-oxygen-voltage (LOV, an algae-/plant-derived photo-activable protein) sensing domain, we achieved a tunable light-induced activation of the FGF signaling pathway in hPSCs. The optically maintained hPSCs in multiple passages have similar cellular and molecular profiles to those cultured with FGF2 protein, without perturbing differentiation capabilities into three germ layers. These results demonstrate that optical stimulation of the FGF signaling pathway is sufficient to maintain hPSCs.

23

CD34+ MCSCS Represent A Subset of Skin-Derived Precursor Cells in The Skin

*Li Pan[1] *, Sandeep S. Joshi[1], Bishal Tandukar[1], Emmanual Kalapurakal[1] and Thomas J. Hornyak[1,2,3]*

[1]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore MD; [2]Research & Development Service, VA Maryland Health Care System, Baltimore, MD; [3]Department of Dermatology, University of Maryland School of Medicine, Baltimore, MD

Melanocyte stem cells (McSCs) which are located in the hair follicle are responsible for hair pigmentation during hair cycles and are a key component of adult stem cells in the skin. Skin-derived precursors (SKPs) have been isolated and described as a multipotent adult stem cells from rodent and human skin. The SKPs have the capability to give rise to neural crest-derived cells in vitro. Our previous studies have shown that CD34+ McSCs which reside in the CD34+ bulge/lower permanent portion (LPP) region during telogen are neural crest-like stem cells. Based on the similarities between the properties of CD34+ McSCs and SKPs, we hypothesized that CD34+ McSCs represent a subset of SKPs in the skin. To determine this, CD34+ McSCs and SKPs were cultured, differentiated and analyzed for similarities in neural crest derivative marker expression. CD34+ McSCs , SKPs and embryonic neural crest stem cell (eNCSCs) were isolated and grown as spheroids in neural crest medium (NCC medium) or SKP medium for 7 days. Subsequently, cells were differentiated in neural crest or SKP differentiation medium and analyzed for marker expression at early (24 hours) and late (1 week) timepoints. At an early timepoint, analysis revealed that 86% of CD34+ MSCs and 76% of eNCSCs expressed p75/NGFR while only 2% of SKPs expressed p75/NGFR in neural crest differentiation medium. The percentages of nestin-positive and fibronectin-positive cells were similar. At a late timepoint, CD34+ McSCs expressed more glial markers GFAP and CNPase compared to SKPs (44% vs 3% and 31% vs 2%, respectively). However, the percentages of βIII tubulin (Tuj1)–positive neuronal cells and smooth muscle actin (α-SMA)-positive cells were similar. Results were similar when cells were differentiated in SKP differentiation medium. Taken together, the data presented here support the hypothesis that CD34+ McSCs are neural crest-like stem cells and represent a subset of SKPs in the skin.

24

Effect of Trauma on Immunotolerance After Myelinating Progenitor Cells Transplantation in Immunocompetent Myelin Deficient Mice

Rui Wang[1,2], A Robert D Stevens [1,2], Piotr Walczak[1]

[1]Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Cell transplantation-based treatments for neurological disease have been promising, yet transplant rejection remains a major barrier to successful regenerative therapies. New immunomodulation methods that induce specific tolerance have been developed for organ transplantation. Our group and others have shown that long-lasting tolerance to transplanted stem cells can be achieved via monoclonal antibodies blocking co-stimulation signaling in T cells (co-stimulation blockade). This approach opens up opportunity for replacing lost or dysfunctional cells in the brain by allografting. However, it has never been studied whether a delayed neuroinflammatory event such as injury, could break the tolerance of the allografted stem cells? Should that occur it could trigger massive and life-threatening rejection. The aim of this study is to provide a systematic assessment of the efficacy and safety of co-stimulation blockade as a tolerance induction strategy. Mouse glial restricted progenitors (GRPs) were derived from mid-gestation (E13) transgenic fLucPLP-GFP mice. mGRPs were transplanted into P3 immunocompetent shiverer mouse neonates(N=10), while wildtype (C57BL6) neonates were used as the control group(N=10). The monoclonal antibodies (anti-mouse-CD154mAb and Abatacept) were administered intraperitoneally on days 0, 2, 4, and 6 post-grafting. We used bioluminescence imaging (BLI) as a non-invasive method to detect the cell rejection. The open field test was performed to test the behavioral and neurological outcome. Neurological consequences were monitored with MRI. After 3 months the transplanted mice were subjected to traumatic brain injury (TBI). After 28 days of TBI the mice were sacrificed for the histological observation of grafted cells, neuroinflammation (Iba1, GFAP, CD45) and status of neurons (NeuN). Transplanted cells were easily detectable with BLI and the signal intensities gradually reduced over three weeks after transplantation to the baseline level in immunocompetent mice, by contrast in shiverer mice with costimulation blockade the signal was maintained at a relatively high level (1.54-2.83 fold higher compared to baseline). 1 week after TBI the BLI signal significant increased in shiverer group (34-41 fold higher compared to that before TBI), through the over three weeks after injury the BLI signal gradually decreased to plateau at a relatively high level (4.7-6.7 times higher compared to that before TBI level). Histological analysis 28days post-TBI showed more GFP positive cells within the TBI injured hemisphere compared with the contralateral hemisphere(p=0.014). Shiverer mice with mGRPs and costimulation blockade showed a reduction in cortical contusion volume compared with wild-type injured mice [(18.3%±4.7%)vs (29.7%±2.2%), p<0.01]. After TBI activation of microglia and astrocytosis was a significantly greater in the shiverer group compared with the wildtype mice. On the contrary, there was a lower number of CD45+ infiltrating leukocytes in shiverer group compared with wildtype (p<0.01). Open field test showed the trend of increased anxiety-like behavior after TBI in shiverer (p>0.05). In MRI 28days after TBI there was clear evidence of brain injury but no differences were observed between the groups and that included lesion morphology, brain edema or blood-brain barrier opening.

These results demonstrate that co-stimulation blockade enables long-term survival and tolerance to the mGRPs in shiverers. Neuroinflammation (TBI) has rather positive effect on transplanted cells. 28 days after TBI, shiverer mice had reduced infiltration by CD45+ cells, increased astrocytosis and increased activation of microglia compared with wildtype mice.

25

Neuronal Differentiation of Neuronal Stem Cells by Autophagy Induction in Oxidative Environment to Treat Traumatic Brain Injury (TBI)

Chinmoy Sarkar[1], Robert A. Brown[1], Nivedita U. Hegdekar[1], Awad Ola[2], Ricardo Feldman[2] and Marta M. Lipinski[1]

[1]Shock, Trauma and Anesthesiology Research (STAR) Center, Department of Anesthesiology, University of Maryland School of Medicine, Baltimore
[2]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore

Traumatic brain injury (TBI) is one of the major causes of death among young adults accounting for almost 30% of all injury-related death. In non-fatal cases it leads to lifelong neurological impairment among patients mainly due to acute and chronic neuronal loss in the injured brain. Since lost neurons cannot be effectively replenished by any available therapy, implantation of neural stem cells (NSC) at the injury site of brain is a promising therapeutic approach to treat TBI. However, highly oxidative and inflammatory environment at the site of injury limits the beneficial effect of NSC transplantation, since such condition is detrimental for NSC survival and differentiation into neurons. Autophagy, a cellular degradative process that removes toxic and damaged macromolecules and organelles, plays important role in neurogenesis. It is also involved in maintaining intracellular ROS homeostasis. However, we observed impairment of autophagy flux in the cortical neurons after controlled cortical impact (CCI)-induced brain injury in mice. Thus the environment at the injury site is highly toxic and not conducive for NSC survival and their differentiation into neurons. We hypothesized that autophagy upregulation in NSCs may protect cells against oxidative insult and will promote their neuronal differentiation in the oxidative environment at injury site. Accordingly, we transduced NSC with FIP200, a component of autophagy inducing Ulk-1 complex, then exposed them to oxidative environment to assess their survival and differentiation. We observed higher level of neuronal differentiation in iPSC-NSCs expressing FIP200 as compared to the cells expressing vector when cultured in the oxidative condition. This is most likely due to the upregulation of autophagy flux through FIP200 and Ulk1 complex. Thus these results clearly demonstrate that autophagy upregulation is beneficial in promoting NSC survival and differentiation under hostile oxidative environment. Next we transplanted iPSC-NSCs in the injured mouse brain at day 7 after injury and treated the mice either with autophagy inducing drug rapamycin or with vehicle. We observed improved survival and enhanced neuronal differentiation of transplanted neural stem cells in mice treated with rapamycin. Taken together our data showed that the transplantation of NSCs with upregulated autophagy in the injured mouse brain is beneficial for NSCs survival and proper differentiation following transplantation in the mouse brain following TBI.

26

Modelling Pitt-Hopkins Syndrome, An Autism Spectrum Disorder, with Patient Derived Induced Pluripotent Stem Cells

Hiler, DJ.[1], Chen, HY.[1], Hamersky, GR.[1], Wang Y.[1], Rao Sripathy, SR.[1], Soudry, OR.[1], Warner, MC.[1], and Maher, BJ.[1,2,3]

[1]The Lieber Institute for Brain Development, Baltimore, MD 21205; [2]The Solomon H. Snyder Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205; [3]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205

Pitt-Hopkins Syndrome (PTHS) is a rare and relatively understudied autism spectrum disorder that is caused by an autosomal dominant mutation or deletion in the gene transcription factor 4 (TCF4). TCF4 is a basic helix-loop-helix (bHLH) transcription factor that plays a critical role in neuronal development through known interactions with other proneural bHLH proteins. To study the role of TCF4 in neuron development, we have differentiated human cortical neurons from both PTHS patient and control derived induced pluripotent stem cells (iPSCs). Using whole-cell patch clamp, immunohistochemistry, qPCR, RNA-seq, and imaging analysis, we have created a robust and quantifiable pipeline to assess the developmental and maturation of PTHS derived-human cortical neurons. With this pipeline, we analyzed IPSC-derived cortical neurons from 6 PTHS patients and 5 control patients and have identified an excitability phenotype in PTHS neurons when compared to controls. Our aim is to use this discovery to identify any potential receptors and/or ion channels that contribute to this phenotype and to identify novel molecular pathways for drug targeting.

27

Modeling Heart Chamber Development and Disease in Cardiac Organoids

Peter Andersen, Chlan Kwon

Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205 USA.

The heart develops from two molecularly and morphologically distinct pools of cardiac progenitor cells (CPCs), referred to the first and second heart field (FHF and SHF), which give rise to different anatomical structures of the heart. Several types of inherited and congenital heart disease are restricted to regions of the heart arising from either the FHF or SHF, thus abnormal CPC development is closely associated with the etiology of chamber-specific abnormalities. While numerous loss- and gain-of-function studies have demonstrated the importance of the various pathways and signals during early heart development and formation, their precise roles in specification and allocation of the FHF and SHF remain to be determined. Here, we developed a PSC-based cardiac organoid system, wherein heart field specification and development can be monitored by green and red fluorescent protein (GFP and RFP) reporters, and for the first time demonstrate how the earliest steps of heart development and formation can be recapitulated and modeled in vitro. These findings set the stage for a mechanistic exploration of heart field specification and development, and to develop a platform for studying chamber-specific development and disease in human PSCs.

28

Neurons from Human Neural Progenitor Transplants Establish Functional Connections in The Adult Cortex: An Optogenetic Study

Jiwon Ryu, Philippe Vincent, Leyan Xu, Nikolaos Ziogas, Richard Sima, Nicholas Stewart, John Curtin, Shirin Sadeghpour, Sascha du Lac, Elisabeth Glowatzki, Vassilis E. Koliatsos

Johns Hopkins University, School of Medicine, Baltimore, MD, USA

We propose that optogenetically engineered human neural progenitors (hNPs) is a great tool in regenerative neuroscience because their integration within the host nervous system and functionality of their differentiated neuronal progenies can be assessed as response to light. In fact, this same response may eventually be leveraged for clinical purposes in regenerative neurology. Here, we transduced H9 embryonic stem cell (ES)-derived hNPs with a lentivirus that harbored human channelrhodopsin (hChR2) and differentiated them into a forebrain lineage. We then characterized their fate and optogenetic functionality of hChR2-hNPs in vitro with electrophysiology and immunocytochemistry. We then transplanted ChR2-hNPs into the motor cortex of normal mice and rats or subjects exposed to traumatic brain injury. We analyzed the survival and neuronal differentiation of hChR2-hNPs by immunohistochemistry followed by stereological analysis. In vitro, hChR2-hNPs demonstrated neuronal phenotypes after 70 days of coculturing with astrocytes. Progressively, they displayed both inhibitory and excitatory neurotransmitter signatures. ChR2 (+) cells from these cultures generated spontaneous action potentials 100-120 days into differentiation and their firing activity was clearly and consistently driven by optical stimulation. We also observed that, after light stimulation, neighboring non-ChR2 neurons generated postsynaptic excitatory and inhibitory responses, evidence that hChR2-hNP-derived neurons established functional connections with other neurons in culture. Three months after transplantation into motor cortex of injured or sham subjects, 60-70% of hChR2-hNPs manifested neuronal cytologies and phenotypes, whereas 60% of cells also expressed ChR2. Depending on depth of inoculation, transplant-derived neurons extended axons through the corpus callosum and the internal capsule and generated terminal fields either in sensorimotor cortical areas lateral to the transplant (in the case of superficial injections through layer 3) or, via the external capsule, in the claustrum, endopiriform area, and corresponding insular and piriform cortices (in the case of deeper injections in layer 5/6 and the adjacent corpus callosum). At these sites, they established synaptophysin (+) terminals, approximately 10% of which expressed GABAergic markers. There was no apparent difference in engraftment, differentiation, or connectivity pattern between injured and sham subjects. We are now analyzing the optogenetic responses of terminal fields in cortex with c-Fos expression and optogenetic field potential recordings after light stimulation of the transplants. Our findings show that we can engineer hNPs with optogenetic properties that are transferred to their differentiated neuronal progenies. Using these properties, we show that neurons derived from hNPs have the capacity of establishing functional synapses with postsynaptic neurons. We conclude that optogenetically endowed hNPs hold great promise as tools to explore new circuit formation in the brain and, in the future, perhaps launch a new generation of neuromodulatory therapies.

29

Using Stem Cells to Repair Radiation-Induced Injury After Tumor Eradication – A Perspective

Chengyan Chu[1], Catherine M. Davis[2], Esteban Verlade[3], Anna Jablonska[1], Miroslaw Janowski[1], Jeff W.M. Bulte[1], Mihoko Kai[3], Piotr Walczak[1]

[1]Russell H. Morgan Dept. of Radiology and Radiological Science, [2]Dept. of Psychiatry and Behavioral Sciences, [3]Dept. of Radiation Oncology, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Gliomas are the most common and deadliest primary brain tumors. Radiotherapy is an adjuvant component of most treatment strategies. Unfortunately, standard fractionated radiation has limited efficacy with a high rate of reoccurrence and results in severe adverse effects. The median survival of patients suffering from glioblastoma is only up to 14.6 months. Clearly there is a dire need for a breakthrough treatment. Oligodendrocytes are characterized by a continuous turnover and likely most affected by radiation. Recent progress in regenerative medicine has shown that they can be replaced by transplantation of glial progenitors, opening up the opportunity for increasing the maximum acceptable radiation dose if injury to oligodendrocytes can be repaired. Here, we explored the feasibility to apply single high dose radiotherapy (40Gy) to eradicate the tumor and rescue the injured brain afterward. We demonstrate that high-dose radiotherapy is efficient to achieve complete eradication of 9L glioma within 4 weeks, as evidenced by MRI and BLI. Longitudinal MRI did not detect any abnormalities up to 10 weeks post treatment; however, behavioral impairment as measured by a recognition memory test was present. On histology, there was only modest evidence of neuroinflammation with mild ipsilateral astrocytosis and activation of microglia. In addition, there was mild demyelination on the side of irradiation but no hemorrhage. Thus, our work shows the feasibility of escalating radiation dose as there is a long-time window between delivering radiation therapy and onset of radiation-induced damage. This provides an opportunity to prevent or repair damage based on myelin as one of the key targets.

Supported by: R01NS102675, 2017-MSCRFD-3942

30

Safety and Efficacy of Intravenous Autologous Mesenchymal Stem Cells for Subacute Ischemic Stroke: A Phase 2 Randomised Controlled Trial

Thomas A. Zeffiro MD, PhD[1], Assia Jaillard MD PhD[2] , Prof. Marc Hommel MD[2], Anaick Moisan PharmD PhD[3] , and Prof. Olivier Detante MD PhD[4] (for ISIS-HERMES Study Group).

[1]University of Maryland Medical Center, Department of Radiology, Baltimore, USA; [2]Univ. Grenoble Alpes, AGEIS, EA 7407, France; [3]Cell Therapy and Engineering Unit, EFS Rhône Alpes Auvergne, Saint Ismier, France; [4]Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, France

Importance: While preclinical studies have demonstrated that mesenchymal stem cells (MSCs) promote functional recovery after stroke, randomized controlled trials (RCTs) using MSCs have raised safety issues. The very few RCTs that have been carried in subacute stroke reported safety, but low feasibility and no significant efficacy of MSC. Objective: To assess safety, feasibility, and explore efficacy of intravenous autologous bone marrow-derived MSCs in patients with moderate-severe subacute stroke. Design: The ISIS-HERMES trial was a single center Phase 2, single blind RCT with two year follow-up. Setting: Patients were recruited between August 31, 2010 and August 31, 2015 at Grenoble-Alpes University-Hospital, France. Participants: We enrolled patients aged 18-70 years with an anterior ischemic stroke, less than two weeks post-onset, with National Institutes of Health Stroke Scale (NIHSS) > 7 at treatment time. Intervention: Patients were randomized 1:1:1 to receive100 million MSCs, 300 million MSCs, or no MSCs intravenously one month after stroke. Real-time dynamic randomization was used, stratifying by lesion side, age and NIHSS. Main Outcomes and Measures: Primary outcomes assessed six-month feasibility and safety, based on the proportion of adverse events. Secondary efficacy outcomes assessed global and motor behavioral recovery. Passive wrist movement fMRI activity from primary motor cortex (MI) was assessed as a physiological outcome measure. A per-protocol analysis was performed to compare 'treated' and 'control' groups. Results: Of 31 enrolled patients (median age (IQR) = 53 years (46-59); 22 males), 7 received 100 million MSCs and 9 received 300 million MSCs. Feasibility was 80%. The two dose groups were merged for comparison with controls. There were 6 adverse events in treated patients and 9 in controls (p=0.19). We observed no effects of MSCs on Barthel Index, NIHSS and modified-Rankin score, but significant improvements in motor-Fugl-Meyer score (t=2.43, p=0.019), motor-NIHSS score (t=2.272, p=0.024) and task-related MI fMRI activity (t=2.67, p=0.009).Conclusions and Relevance: Intravenous autologous MSC treatment was safe and feasible. Brain activity biomarker results suggest that MSC treatment improve motor system recovery. The observed benefits might result from brain repair through MSC paracrine effects. MSC beneficial effects in subacute stroke need to be confirmed by further studies. Trial Registration: ClinicalTrials.gov Identifier NCT00875654.

31

Generating 3D Brain Organoids With Endothelial Cells From Human iPSCs

Anna Bagnell, Marie Medynets, Tongguang Wang

Neurodifferentiation Unit, Translational Neuroscience Center, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

In vitro models are important for understanding the mechanisms underlying human neurological disease pathologies and the development of therapeutics. Compared with traditional monolayer of mixed neuronal cultures, 3D brain organoids cultures based on induced pluripotent stem cells (iPSCs) contain advanced cell-cell interactions and organ- level structures and functions central to many disease etiologies. Evidence indicate that blood vessels and endothelial cells as part of human brain play important roles in human brain development and neurodegeneration. However, it is still difficult to develop organoids containing both endothelial and neural cells as they are from different germ layers.

We hypothesized that inducing iPSC-derived embryoid with endothelial differentiation medium at early stage followed by neural induction may help generating 3D cerebral organoids with mixed neuronal and endothelial cells. To do that, protocols were designed and optimized to permit the differentiation of human iPSC embryoid bodies into both neuronal and endothelial cell types concurrently. Gene expression analysis and immunostaining of spheroids indicated the presence of the two cell types. Future studies will look at standardizing the models to make it useful for study neurological disorders such as ALS.

32

Role of Neurotoxic Astrocyte in Neurodegenerative Diseases

Tae-In Kam[1,2,3*], *Hyunhee Kim*[1,2,3]*, *Jinchong Xu*[1,2,3], *Hyejin Park*[1,2,3], *Aanishaa Jhaldiyal*[1,2,3], *Sung Ung Kang*[1,2,3], *Ted M. Dawson*[1,2,3,4,5], *Valina L. Dawson*[1,2,3,5,6]

[1]Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA [2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; [3]Adrienne Helis Malvin Medical Research Foundation, New Orleans, LA 70130-2685, USA; [4]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; [5]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA [6]Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Activation of microglia by classical inflammatory mediators can convert astrocytes to a neurotoxic A1 phenotype in murine cultures. Although activated microglia and astrocytes expressing A1 markers have been identified in the brain from patients of neurological disorders including Alzheimer's disease (AD) and Parkinson's disease (PD), their role is poorly understood. Here we report that human microglial-like cells and astrocytes can be differentiated from H1 human embryonic stem cells (hES). The mRNA transcriptome analysis demonstrates that they express highly specific microglial and astrocytes markers, respectively. These hES-derived microglia and astrocytes have the phenotype, gene expression profile and functional properties of these cell types. Human ES-derived microglial-like cells are activated and secrete a variety of cytokines by *a*-synuclein preformed fibrils (PFF) and Aβ oligomers. The treatment of activated microglia conditioned medium (MCM) containing TNF*a*, C1q and IL-1*a* to human astrocytes induces conversion of A2 astrocyte to A1 astrocyte. Furthermore, A1 astrocyte conditioned medium (ACM) induced by *a*-syn PFF and Aβ oligomers causes the death of human dopaminergic neurons and cortical neurons, respectively. Together, hES-derived microglial-like cells and astrocytes can be used to study the contribution of neurotoxic A1 astrocytes in neurodegenerative disorders, providing opportunities for the development of new treatments for neurological disorders.

33

Development of Imaging Biomarkers for Stem Cell Transplantation in Amyotrophic Lateral Sclerosis

Jean-Philippe Richard[1], *Uzma Hussain*[1], *Sarah Gross*[1], *Arens Taga*[1], *Mehreen Kouser*[2], *Akshata Almad1*, *James Campanelli*[3], *Jeff W.M. Bulte*[2] *and Nicholas J. Maragakis*[1]

[1]Johns Hopkins University School of Medicine, Department of Neurology, Baltimore, MD; [2]Johns Hopkins University School of Medicine, Department of Radiology and Institute for Cell Engineering, Baltimore, MD; [3]Q-Therapeutics, Inc. Salt Lake City, UT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease where motor neurons progressively degenerate and die. Astrocytes and other glial subtypes participate in ALS progression and are therefore targets for therapeutic strategies. Previously, our lab investigated the potential of human Glial Restricted Progenitor Cells (Q Cells) to provide neuroprotection in ALS animal models. The development of Q-Cells as a cellular therapeutic for ALS has shown significant progress. An FDA IND for the use of Q Cells has been granted for our planned clinical trial delivering these cells to the spinal cord of ALS patients. One of the challenges in transplantation therapeutics in the CNS is the absence of data on monitoring survival and migration of the cells after transplantation in the living patient. Understanding how Q Cells behave after transplantation will provide critical information that can help us optimize the number of cells for transplantation, understand migratory patterns inside the spinal cord, and monitor cell survival over time. In this study, we will use MRI-based techniques to examine cell survival and migration over time following the transplantation of CS-1000 labeled Q-Cells into rodent models of ALS. CS-1000 is a fluorocarbon-based (^{19}F) emulsion that allows *in vitro* labeling of Q Cells for later studies of cell tracking using MRI. CS-1000 can also incorporate fluorescent tags (DM Red or DM Green) to allow for immunocytochemistry. Important for its clinical use, we now have extensive data characterizing Q Cells following the incubation with CS-1000. We have been able to determine the sub cellular localization of the fluorinated compound and have performed several experiments to optimize the dose of CS-1000 tolerated by Q cells and the duration for incubation to provide the maximum loading effect. Importantly, we have also shown the absence of any statistically significant detrimental effect of CS-1000 on the differentiation of Q Cells into relevant glial cell subtypes. To demonstrate that our labeled cells were capable of survival, integration and differentiation into astrocytes following transplantation into the spinal cords of mice, we transplanted Q cells labeled with DM Green CS-1000. We show that these cells can be targeted to the ventral horn and appropriately differentiate into astrocytes. Importantly, one week after transplantation, we did not see any uptake of the DM Green CS-1000 labeling by host mouse cells. Consequently, these data demonstrate that the CS-1000 labeling will give us a true assessment of transplanted cells without concern for false positive labeling of host neural cells. In parallel, we conducted ^{19}F NMR experiments on CS-1000 labeled QSV40 cells (an immortalized Q cell line) in order to quantify the CS-1000 uptake by the labeled cells. NMR Spectra and ^{19}F content analysis shows that increasing the CS-1000 concentration in the labeling media increases the ^{19}F content per cell. Having optimized the in vitro and NMR paradigms, MRI studies in rodents receiving CS-1000 labeled Q Cells into the CNS are underway. Taken together, we are generating pre-clinical data necessary for demonstrating the safety and clinical utility of CS-1000 in preparation for translational use in patients.

34

High-Throughput Methods for Evaluating Cellular Phenotypes and Neural Activity in Human Induced Pluripotent Stem Cell (hIPSC)-Derived 3D-Organoids

***Madel Durens**, Andre W. Phillips, Jonathan E. Nestor, Jason W. Lunden, Omotayo Oduwole, Michael W. Nestor*

The Hussman Institute for Autism, Baltimore, MD 21201, USA

The etiology of neurodevelopmental conditions including autism spectrum condition (ASC) is often complex. Genetic and environmental heterogeneity in individuals with ASC has not only made it difficult to study underlying biology but has limited the translatability of animal models. Human induced pluripotent stem cell (iPSC) technology provides a platform for examining potential cellular phenotypes wherein the individual genetic background is conserved. More recently, the limitations of traditional 2-dimensional (2D) cultures in reflecting tissue complexity have led to efforts to develop more complex 3-dimensional (3D) cultures that can more faithfully mimic aspects of early neurodevelopment. However, issues with variability, reproducibility, and sufficient statistical power have also restricted the utility of 3D models. To address this, high content screens that allow for larger sample sizes with greater efficiency have to be adapted to 3D models. In this study, we used the 3D serum free embryoid body (SFEB) model optimized by Nestor et. al., (2013), a model for early corticogenesis, and applied several high content assays to probe for phenotypes relevant to ASC. SFEBs were differentiated from iPSCs by plating in a 96-well V-bottom plate for 14 days, after which the SFEBs were transferred to cell culture inserts and grown until 60 days in vitro (DIV). First, we used a 48-well multi-electrode array (MEA) to measure spontaneous local field potentials, as well as responses to various pharmacological agents. We also demonstrated synaptic plasticity in the SFEBs by chemically inducing long-term potentiation. To visualize neuronal activity in individual cells we transduced the SFEBs with AAV9-GCamp6, and imaged calcium activity after one week. We imaged and analyzed calcium signaling using the ThermoFisher ArrayScan XTI to evaluate the applicability of this method to high-content analysis. Next, we evaluated neuronal morphology by coupling standard immunolabeling with Map2 and analyzing the images using the neurite detection protocol on the ArrayScan. Lastly, because excitatory/inhibitory imbalance has emerged as one possible hypothesis that underlies ASC neurobiology, we evaluated the ratio of γ-aminobutyric acid (GABA)ergic and glutamatergic neuronal populations within the SFEBs by immunolabeling with GABA and the glutamate transporter VGLUT. We establish here that these cell populations can be analyzed in SFEBs using a high content screen. Altogether, we aim to develop a platform to establish neuronal phenotypes in 3D cultures, and use these methodologies for phenotypic drug screening.

35

Combinatorial Polymer Matrices Enhance *In Vitro* Maturation Of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

***Young Wook Chun**[1] , and Charles C. Hong[1,2]*

Division of Cardiovascular Medicine, University of Maryland School of Medicine, Baltimore, MD

Cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) hold great promise for modeling human heart diseases. However, iPSC-CMs studied to date resemble immature embryonic myocytes and therefore do not adequately recapitulate native adult cardiomyocyte phenotypes. Since extracellular matrix plays an essential role in heart development and maturation in vivo, we sought to develop a synthetic culture matrix that could enhance functional maturation of iPSC-CMs in vitro. In this study, we employed a library of combinatorial polymers comprising of three functional subunits - poly-ε-caprolacton (PCL), polyethylene glycol (PEG), and carboxylated PCL (cPCL) - as synthetic substrates for culturing human iPSC-CMs. Of these, iPSC-CMs cultured on 4%PEG-96%PCL (each % indicates the corresponding molar ratio) exhibit the greatest contractility and mitochondrial function. These functional enhancements are associated with increased expression of cardiac myosin light chain-2v, cardiac troponin I and integrin alpha-7. Importantly, iPSC-CMs cultured on 4%PEG-96%PCL demonstrate troponin I (TnI) isoform switch from the fetal slow skeletal TnI (ssTnI) to the postnatal cardiac TnI (cTnI), the first report of such transition in vitro. Finally, culturing iPSC-CMs on 4%PEG-96%PCL also significantly increased expression of genes encoding intermediate filaments known to transduce integrin-mediated mechanical signals to the myofilaments. In summary, our study demonstrates that synthetic culture matrices engineered from combinatorial polymers can be utilized to promote in vitro maturation of human iPSC-CMs through the engagement of critical matrix-integrin interactions.

36

Innovative Applications of 3D Tissue Engineering Technology

***Ha Nam Nguyen, PhD** ; 3Dnamics, Inc. Baltimore, MD, USA*

The quest to find novel therapeutics for human disorders has been hindered by the lack of access to relevant experimental models. Conventional monolayer cell culture and animal models often do not fully recapitulate genuine disease phenotypes and are poor predictors of how drugs will work in humans. Thus, majority of preclinical drug testing do not translate to clinical success and have a high failure rate. However, recent progress in 3D tissue engineering technology offers a promising platform that may be the key in reversing current drug developmental trend and bridging the gap between conventional systems and humans. These 3D tissues, derived from pluripotent stem cells (PSCs), can mimic architecture composition, genetic profile, and physiology of those organs found *in vivo*. Such 3D tissues provide unique tools for studying organogenesis, generation of clinically relevant cell or organoid types, and development of new disease models and pharmacotherapy. Here, we show that PSC-derived 3D brain tissues can be used to model neurological disorders, including Parkinson's disease and Alzheimer's disease, and to perform drug/toxicity screen.

37

Integrating Motion Correction into CEST MRI pH Imaging

KowsalyaDevi Pavuluri , Changlin Li*, Michael T. McMahon*#*

**Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine; #F. M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Research Institute, Baltimore, Maryland 21205, United States*

Introduction: Chemical exchange saturation transfer (CEST) imaging is a highly versatile MRI contrast mechanism well suited to performing pH imaging. To produce pH images suitable for monitoring stem cell transplants, an MRI acquisition scheme is used to collect a series of saturation transfer images over several minutes on exogenous CEST agents dispersed in hydrogels supporting the stem cell grafts in living subjects. Breathing related image artifacts are present in the resulting images which must be taken into account in the post-processing. In this study, we identify several promising image registration methods, integrate these into our CEST post-processing schemes and evaluate their performance in mice. Mesenchymal stem cells (MSCs) naturally differentiate into connective tissue and are thus attractive for facilitating the repair of intervertebral discs in the back. Determining how long the engrafted MSCs survive is important to determine if the procedures and scaffold materials should be amended. In our previous studies we have developed a method to monitor transplanted cell survival using CEST MRI pH imaging. One of the challenges is that the image acquisition times are relatively long (several minutes) compared to breathing rates, which results in artifacts, and as a result strategies are required which take motion into account during image post-processing. The respiration rate may fluctuate during a single imaging session, we have observed this varying from 30 times/min to even 60 times/min for mice. We have tested three options for motion correction: (a) Normalized mutual information (NMI) based rigid registration; (b) Normalized Cross Correlation based rigid registration; and the (c) Demons algorithm based non-rigid registration. Results: Live animal experiments consisted of administering iopamidol at a weight controlled dose of 1.5 g/kg and acquired saturation transfer data on a single slice containing the center of both kidneys. We acquire four images prior to administration to allow for a good reference image, with the amount of contrast in the kidneys building up over 30 minutes and fairly uniform across both kidneys, which is nice for testing our motion corrections. Aiming to measure the similarity between the reference image and after-registration, we use cross correlation as the similarity index. Correlation coefficient directly reflects the structural similarity between two images but doesn't take intensity into account, which is required in this case because the intensity can change based on inflow of contrast material. The NMI, NCC and demons algorithms were tested on images with 48x48 resolution with correlation coefficients of 0.980 0.982 and 0.990 respectively. Based on this, we concluded that the demons algorithm worked the best. We then compared this algorithm using both the original 48 x 48 resolution and an interpolation based 256 x 256 resolution with correlation coefficients of 0.990 and 0.997 respectively. Visual inspection also indicates obvious improvement in the resulting aligned image series. We have further evaluated this by drawing an ROI enclosing a single kidney, and comparing the change in contrast with time for this ROI both pre- and post- registration. The resulting curves displayed minimal differences in contrast buildup with time, further indicating the suitability of this method. In summary, based on these findings the demons non-rigid registration algorithm can readily be integrated into CEST post-processing schemes and is suitable for correcting motion artifacts.

Acknowledgement:This study is funded by MD Stem Cell Research Fund.

38

GMP-Compliant Non-Viral CRISPR-Mediated Mutation Correction in Patient CD34+ Cells with Sickle Cell Disease (SCD) Achieves 80% Wild Type Adult Hemoglobin Expression in Differentiated Erythrocytes

Linhong Li[1] , Naoya Uchida[2]*, Juan Haro Mora[2], Selami Demirci[2], Lydia Raines[2], Cornell Allen1, Suk See De Ravin3, Harry L Malech[3], Madhusudan V. Peshwa[1], John F Tisdale[2]*

*[1]MaxCyte, Inc., Gaithersburg, MD; [2]Sickle Cell Branch, NHLBI, NIH, Bethesda, MD; and [3]Laboratory of Clinical Immunology and Microbiology, NIAID, NIH, Bethesda, MD.*Equal contribution*

The c.20A>T mutation of β-globin gene causes sickle cell disease (SCD). Allogeneic hematopoietic stem cell (HSC) transplantation can cure SCD, but most lack a suitable donor. Ex vivo gene therapy strategies, including lentiviral mediated gene transfer or endonuclease mediated BCL11a knockdown allowing fetal hemoglobin (Hb) induction, are currently under evaluation. Correction of the SCD mutation by non-viral gene editing of autologous HSCs would add an alternative strategy and permit endogenous gene expression at its natural regulatory locus and the beneficial reduction of the pathogenic sickle Hb production. Allogeneic transplantation has established that the therapeutic threshold for clinical benefit is ≥20% donor chimerism. We previously reported efficient correction of a monogenic "hotspot" mutation in the CYBB gene in X-linked chronic granulomatous disease (CGD) patient HSCs with a robust, scalable, cGMP, and regulatory compliant process (Sci Transl Med 2017) that we now apply to SCD. In initial studies using a B cell line (B-LCL) created from SCD patient and healthy volunteers' CD34+ HSCs, we developed a SCD mutation specific guide RNA, and a normal β-globin specific guide RNA (converse). The converse guide differed by only one nucleotide from the SCD mutation specific guide, where each guide could be used together with a single stranded DNA donor to effectively alter the wild type to SCD and the SCD to wild type, respectively (ASGCT 2017). At first, we optimized homology directed repair (HDR) at the SCD locus by integrating a HindIII enzyme site. We observed efficient site-specific insertion of the HindIII-marker in the B-LCL as evidenced by HindIII digestion of the PCR products (~50%), and targeted sequencing (~35% HDR and ~50% Indel). The optimized process was applied to correct SCD CD34+ HSCs to achieve similar biallelic HDR rates for HindIII site insertion as well as gene correction from the SCD mutation to the normal β-globin sequence (up to ~35% correction and ~50% Indel). Interestingly, this correction was maintained during erythroid differentiation in culture. Among erythrocytes differentiated from corrected SCD CD34+ cells in vitro, wild type adult Hb protein levels were above 60% as assayed by both reverse phase HPLC and Hb electrophoresis, and sickle Hb production decreased from 100% to 20% after correction. In summary, based on these in vitro correction rates confirmed by targeted sequencing, wild type adult Hb protein expression, and substantially decreased sickle Hb amounts, we are starting to evaluate engraftment of corrected SCD patient HSCs in immunodeficient mice. The high rate of engraftment in immunodeficient mice of similarly corrected HSCs observed in our published CGD study puts these results observed for in vitro correction of SCD within the therapeutic window of reversing SCD.

39

Mechanical Characterization of Engineered Cardiac Tissues for Clinical Application

Rachana Mishra, University of Maryland, Baltimore, MD

Background: Hypoplastic left heart syndrome (HLHS) patients have the highest morbidity and mortality rates of all congenital heart disease (CHD) patients in spite of significant medical and surgical advances. The high HLHS mortality is due to pressure/volume overload of the single right ventricle (RV) leading to its dysfunction and failure even after surgery palliation. We have already showed that administration of MSCs in neonatal swine model of RV pressure overload. Improved the RV functions but the mechanism underlying the beneficial effects of MSCs on RV function is unclear. Hypothesis: We hypothesize that injected MSCs improves RV function by secreting exosomes enriched with growth differentiation factor 15 (GDF15) and miR-132, which synergistically activate a novel GDF15-miR-132-Smad2/3 signaling pathway in cardiomyocytes this leads to positive cardiac remodeling response. Methods: This study was performed using protocols that were reviewed and approved by our Institutional Animal Care and Use Committee and following the 1996 Guide for the Care and Use of Laboratory Animals. Immunosuppressed Yorkshire swine (6–9 kg, 14–21 days of life) underwent pulmonary artery (PA) banding followed by injection of MSCs or placebo at 30 min postbanding. Echocardiography was performed to assess structural and functional changes at baseline, postbanding at 4 wk. Standard RNA interference technology to KD GDF15, miR-132 and miR-21expression. Results and Conclusion: We have already shown that four weeks after PA banding in swine, end-systolic and end-diastolic areas were preserved in the MSC-injected group compared to baseline while the placebo group demonstrated significant pathologic RV dilation ($P < 0.01$), Similarly, RV function was preserved in the MSC-treated group ($P < 0.01$). Here we are reporting a novel finding that injected MSCs lead to positive remodeling in pressure overloaded swine myocardium by decreasing the levels of cellular miR-21 and releasing exosomes enriched in GDF15 and miR-132. Identification of upstream regulators and downstream effectors of the GDF15-miR132-Smad2/3 pathway will be critical for the clinical success of this therapy.

40

Transplanted Volar Fibroblasts to Nonvolar Skin Induce Ectopic Volar Skin

Sooah Kim, Dongwon Kim*, Seakwoo Lee, Vicky Prizmic, Benjamin Evans, Luis A. Garza*

Dept. of Dermatology, Johns Hopkins School of Medicine, Baltimore, MD

Amputees have skin problems at stump sites due to the long-term use of prosthetics. The conversion of thin skin at stump sites to palmo-plantar (volar) type skin with friction, irritation and pressure resistance can be one solution for these skin problems. In previous study, we demonstrated that KERATIN9 (KRT9) is the most uniquely expressed gene in volar epidermis compared to nonvolar and its expression can be induced in nonvolar keratinocytes by volar fibroblasts. As a preclinical study, in this study, we transplanted mouse volar fibroblasts into non-volar skin area to investigate the conversion of skin identity by evaluating Krt9 expression. Donor volar fibroblasts were isolated from paw of Luciferase positive/GFP positive (LUC-eGFP) transgenic mice and then injected into recipient nonvolar ears of immunodeficient mice (NSG). Luciferase activities of donor injected fibroblasts were quantified using IVIS Lumina II imaging system. Finally, the recipient ears were collected and analyzed by qRT-PCR and immunostaining to evaluate Krt9 expression 8 weeks after transplantation. The luciferase signal of one million volar fibroblast cells significantly decreased during the first 2 weeks after transplantation such that only 10% of injected fibroblast survived for more at 2 months post-injection. For preventing this decay, we attempted to divide dosing of the same total amount of cells separated into multiple injections and inject single injection. The luciferase activities of fibroblast did not show a similar decay in multiple injection and single injection. Finally, we collected tissues from these treatments and found that Krt9 expression level and epidermal thickness increased 4.8-fold and 1.8-fold, respectively, in ears injected with volar fibroblasts compare to vehicle ($n=3$ and $p<0.05$). These findings demonstrate that multiple injections could improve the survival and the regulation of injected site-specific fibroblasts for converting skin identity. Therefore, we suggest that the conversion of skin identity with the injection of volar fibroblasts to the stump site of amputees may improve the prosthetic use and the quality of life for amputees. Furthermore, we expect our research will contribute to develop both basic and translational study to advance the knowledge and technology in the stem cell therapy. *Funding: Maryland Stem Cell Research Fund awarded to Dongwon Kim*.

41

A Self-Healing Hydrogel as An Injectable Instructive Carrier for Cellular Morphogenesis

Zhao Wei, Sharon Gerecht

Department of Chemical and Biomolecular Engineering and Materials Sciences and Engineering, Johns Hopkins University, Baltimore, MD

Transplantation of progenitor cells can accelerate tissue healing and regenerative processes. Nonetheless, direct cell delivery fails to support survival of transplanted cells or long-term treatment of vascular related diseases due to compromised vasculature and tissue conditions. Using injectable hydrogels that cross-link in situ, could protect cells in vivo, but their sol-gel transition is time-dependent and difficult to precisely control. Hydrogels with selfhealing properties are proposed to address these limitations, yet current self-healing hydrogels lack bio-functionality, hindering the morphogenesis of delivered cells into a tissue structure. Here we establish a gelatin (Gtn)-based self-healing hydrogel cross-linked by oxidized dextran (Odex) as an injectable carrier for delivery of endothelial progenitors. The dynamic imine cross-links between Gtn and Odex confer the self-healing ability to the Gtn-I-Odex hydrogels following syringe injection. The self-healing Gtn-I-Odex not only protects the progenitors from injected shear force but it also allows controllable spatial/temporal placement of the cells. Moreover, owing to the cell-adhesive and proteolytic sites of Gtn, the Gtn-I-Odex hydrogels support complex vascular network formation from the endothelial progenitors, both in vitro and in vivo. This is the first report of injectable, self-healing hydrogels with biological properties promoting vascular morphogenesis, which holds great promise for accelerating the success of regenerative therapies.

42

Immunosuppression Increases Regenerative Potential of Adult Rat C-KIT+ Cardiac Progenitor Cells In Allogeneic Rat Myocardial Infarction Model

Muthukumar Gunasekaran , Rachana Mishra, Sudhish Sharma, Progyaparamita Saha, Mir Yasir Arfat, Chetan Ambastha, David Morales, Gregory J Bittle, Deqiang Li, Sunjay Kaushal

University of Maryland, Baltimore, MD

Rationale: Cardiac progenitor cells (CPCs, c-kit+) are well-characterized stem cell type shown to potentiate cardiac regeneration in myocardial infarction (MI) in adults and pre-clinical models. However, the innate immune response directed to transplanted adult CPCs (aCPCs) reduces cell retention and MI recovery. We aimed to increase the regenerative potential of aCPCs by reducing the immune response directed to the transplanted aCPCs using cyclosporine A (CSA). Methods: Adult CPCs (aCPCs) were isolated from Wister Kyoto rat was transplanted (1X106) to Brown Norway MI rats. Immune suppressant CSA were administered orally to MI rats with rCPCs transplantation group (aCPCs+CSA). CSA alone and Iscove's Modified Dulbecco's Media (IMDM) served as control groups. Echocardiogram was performed to determine MI recovery on day 1, 7 and 28. The transplanted rCPCs cell retention was detected using GFP expressing rCPCs on heart tissue by immune-histochemistry (IHC). The rate of apoptotic cell death on injured myocardium was measured using TUNNEL assay. The inflammatory CD-68+ cells were measured by IHC. Sera collected on day 2, 7, 14, 21 and 28 were utilized to measure the inflammatory (IL-2, IL-17, IFN-γ, TGF-β), anti-inflammatory (IL-10) cytokines and antibodies against cardiac self-antigens (SAgs) Troponin-T and Myosin. Results: MI rats transplanted with aCPCs+CSA demonstrated significant increase in MI recovery and cell retention compared to MI rats with aCPCs, CSA and IMDM controls ($p<0.05$). MI rats transplanted with aCPCs showed increased infiltration of CD-68+ cells and apoptotic cells in the infarcted myocardium compared to aCPCs+CSA group ($p<0.05$). Sera collected on day 2 and 7 of aCPCs group showed increased inflammatory cytokines, antibodies to cardiac SAgs and reduced anti-inflammatory cytokines. In contrast MI rats transplanted with aCPCs+CSA showed reduced inflammatory cytokines and increased anti-inflammatory cytokine IL-10 ($p<0.05$). Conclusions: In conclusion, MI rats transplanted with allogeneic aCPCs+CSA showed increased cell retention, reduced inflammatory cells and cytokines compared to aCPCs, CSA and IMDM control. Therefore, CSA reduces inflammation and increases the retention of transplanted aCPCs that resulted in increased myocardial recovery.

43

Rapid and Efficient Generation of Oligodendrocyte Precursor Cells

Chengchen Hu[1,3], Kimberly Wang[1,3] , Qingfu Xu[1,2,3], Damon Ceylan and Yunqing Li[1,3]

[1]Hugo W. Moser Research Institute at Kennedy Krieger, 707 N. Broadway, Baltimore, MD, USA 21205; Department of [3]Neurology, The Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Baltimore, MD, USA 21287

Currently, generation of induced OPCs(iOPCs) from hiPSCs and their stepwise differentiation into OLs are time-consuming (75-120 days) and inefficient (20-75%) and generating heterogeneous cells (oligodendrocyte and astrocyte) and tumorigenic potential (host genome modifications by using lentiviral constructs), and thus have limitations for regenerative medicine. Here, we have established and validated a robust and efficient protocol that generates expandable iOPCs from hiPSCs within 10 days using innovative transcription factors combined with the neural specification signals. Moreover, with some modifications, this protocol can be used to convert human fibroblasts into OPC-like cells. We demonstrate that combination of transcription factors cocktail (Sox10, Olig2 and ASCL1) and small molecular cocktail induces 80-90% PDGFR+ cells within 10 days. These cells exhibit typical small bi-polar or tri-polar OPC-like morphologies, maintain their self-renewal capacity and express OPC-specific markers NG2, PDGFRα, Sox10 and O4. Moreover, these iOPCs maintain high proliferation as shown by 60% Ki67+ positive. Under mature differentiation condition, these iOPCs became multipolar O4+ cells, and myelin basic protein MBP+ mature oligodendrocytes with complex branches forming. Our protocol offers a unique approach to combine transcription factor cocktail and small molecule cocktail to generate iOPCs from hiPSCs and human fibroblasts without genetic manipulation in a clinically applicable timeframe.

44

Activation of the NLRP3 Inflammasome In Human Dopamine Neurons As A Consequence of Parkin Dysfunction

Nikhil Panicker , Manoj Kumar, Tae-in Kam, Sung Ung Kang, Valina Dawson, Ted M. Dawson

Parkinson's disease (PD) is a chronic, progressive, neurodegenerative disorder that affects over a million people within the U.S. Underlying the clinical symptoms of PD is the degeneration of dopamine (DA) neurons located in the pars compacta of the substantia nigra (SNpc). Inhibition of the E-3 ubiquitin ligase parkin is central to familial as well as sporadic PD progression. One of the major criticisms of PD research is that despite the plethora of studies using rodent or cell culture models, pathologically relevant drug targets have not been hitherto identified because the underlying physiology of these systems differs substantially from that of human DA neurons. To address this issue, we generated human embryonic stem cell (hES) differentiated DA neurons (hDA neurons). We also developed two Parkin knockout hES lines, allowing us to obtain Parkin knockout hDA neurons. Preliminary results from the Parkin KO hDA neurons revealed upregulation of the cytosolic stress receptor NACHT, LRR, and PYD domains-containing protein 3 (NLRP3), and Caspase-1 activation, suggesting that loss of Parkin licenses NLRP3 inflammasome activation in human DA neurons. Further studies indicate that parkin interacts with and polyubiquitinates NLRP3, tagging it for proteasomal degradation. Finally, Immunohistochemistry analysis of PD patient and age-matched control ventral midbrain brain sections shows NLRP3 upregulation and Caspase-1 activation within tyrosine hydroxylase (TH) positive DA neurons under PD conditions. These experiments suggest that unconventional neuronal NLRP3 inflammasome activation may occur following parkin dysfunction. Future work will explore the consequences of neuronal NLRP3 inflammasome hyperactivation in contributing to PD-associated neurodegeneration.

45

Divergent Stem Cell Function Between Skeletal and Soft Tissue CD146+ Human Pericytes

Jiajia Xu[1], Dongqing Li[1, 2], Yiyun Wang[1], Leslie Chang[1], Carolyn A. Meyers[1], Bianca Vezzani[2], Mario Gomez Salazar[2], Yongxing Gao[1], Kristen Broderick[1], Carol Morris[1], Bruno Peault[2,3], Aaron W. James[1]

[1]Johns Hopkins University, MD, 2University of Edinburgh, UK, 3University of California, Los Angeles, CA,

INTRODUCTION: Human pericytes demonstrate multilineage differentiation potential, and their descendants participate in tissue homeostasis and repair. However, increasing evidence from developmental biology suggests that regional specification by tissue of origin exists among human pericytes. Interestingly, and despite their heterologous origin, human adipose-tissue derived pericytes have been used with success to regenerate bone by our research group across pre-clinical models. Clearly some plasticity exists in the reparative phenotype of human pericytes. In sum, understanding how pericytes regenerate their tissue microenvironment with such remarkable fidelity is of central importance for cell-based efforts in regenerative medicine. Here, we sought to define in detail the differentiation potential and plasticity of CD146+ human pericytes from skeletal and soft tissue sources. METHODS: Uncultured CD146+CD31-CD45- pericytes were derived by fluorescent activated cell sorting (FACS) from human periosteum, adipose, or dermal tissue (N=3 patient samples per tissue type). Canonical features of pericytes were assayed, including characteristic pericyte marker expression (qRT-PCR, flow cytometry, and immunocytochemistry), multilineage in vitro differentiation potential (osteogenesis, adipogenesis, chondrogenesis), and paracrine-induced tubulogenesis. Next, quantitative metrics of in vitro osteogenic and adipogenic differentiation were assessed across human tissues of origin. Intramuscular implantation studies assessed ectopic bone formation across human pericyte sources. The transcriptome of human periosteum, adipose, or dermal pericytes was assessed by Clariom D microarray. RESULTS: Pericytes were first visualized within human periosteum as a CD146 expressing perivascular cell population (Fig. 1A). Uncultured human periosteal pericytes were isolated as a CD146+CD31-CD45- cell fraction. Frequency of CD146+ pericytes within the periosteum was 8.17% (7.05% SD), similar to the frequencies of CD146+ pericytes within soft tissue. Next, the comparative ability of pericytes to differentiate down osteogenic and adipogenic lineages was assessed. Periosteal pericytes demonstrated a striking tendency to undergo osteoblastogenesis, while soft tissue pericytes did not. In contrast, adipose pericytes demonstrated a tendency to undergo adipogenesis. Intramuscular implants again recapitulated tissue of origin, with only periosteal pericytes demonstrating neo-osteoblastogenesis. Microarray analysis by principal component analysis and unsupervised hierarchical clustering demonstrated clear differences by anatomic origin, and an enrichment among periosteal pericytes in CXCR4 signaling. DISCUSSION: In sum, skeletal and soft tissue pericytes differ in their relative lineage differentiation potential and ability to form bone. Plasticity exists, however, and manipulation of key signaling pathways may 'coax' a soft tissue pericyte toward an osteoblastogenic cell fate. Ongoing studies are focusing on manipulation of CXCR4 signaling in order to 'rescue' the osteogenic potential of soft tissue pericytes.

46

Location- And Dose-Dependent Attenuation of Experimental Autoimmune Encephalomyelitis Using Glial-Restricted Progenitors

Aline M. Thomas[1], Shen Li[1], Chengyan Chu[1], Irina Shats[1], Jiadi Xu[2], Peter Calabresi[3], Peter van Zijl[1,2,] Piotr Walczak[1,2], and Jeff W.M. Bulte[1,2]

[1]Department of Radiology and Radiological Science and Cellular Imaging Section, Institute for Cell Engineering, Johns Hopkins University School of Medicine; [2] F.M. Kirby Center for functional brain imaging, Kennedy Krieger Institute Kirby Research Center; [3]Department of Neurology, Johns Hopkins University School of Medicine

Introduction: Glia store lactate at imageable levels to supply neurons, which may be a potential target for halting MS progression. We explored the regions of the brain benefited by glial transplantation in a primary progressive experimental autoimmune encephalomyelitis (EAE) animal model for multiple sclerosis MS using lactate-sensitive MRI. Methods: Luciferase+ glial-restricted progenitors (GRPs) were transplanted into naïve or EAE-induced mice at different doses into the motor cortex or lateral ventricle using a stereotaxic device. Lactate was monitored using 1H MRS and on-resonance variable delay multiple pulse chemical exchange saturation transfer (onVDMP CEST) MRI. Cell engraftment and survival was confirmed using bioluminescent imaging (BLI) and histology. Results: onVDMP CEST and 1H MRS (lactate) signals changed spatiotemporally in EAE mice. GRPs enhanced onVDMP CEST and 1H MRS (lactate) signals in cell culture phantoms. onVDMP CEST and 1H MRS (lactate) signals were enhanced in naïve mice and in EAE mice upon GRP transplantation at or proximal to the transplant site. BLI showed cell engraftment varied by transplant site location. Attenuation of paralysis by GRPs in this EAE model for MS was dependent on both the dose and transplant site location.

47

Generation and Selection of Pluripotent Stem Cells for Robust Differentiation to Insulin-Secreting Cells Capable of Reversing Diabetes in Rodents

Sheryl Southard, and William Rust ; Seraxis, Inc.

Induced pluripotent stem cell (iPSC) technology enables the creation and selection of pluripotent cells with specific genetic traits. We developed a pluripotent cell line to form replacement pancreatic cells as a therapy for insulin-dependent diabetes. Beginning with primary pancreatic tissue acquired through organ donation, cells were isolated and re-programmed using non-integrating vectors. Each of the resulting iPSC lines was then subjected to a differentiation protocol to guide the cells to the endodermal and pancreatic fate. The candidate line that most consistently generated highly pure populations of endocrine pancreas precursors was selected for further development. This approach created an iPSC-variant cell line, SR1423, with a genetic profile correlated with preferential differentiation toward endodermal lineage at the loss of mesodermal potential. An improved differentiation protocol, coupled with SR1423, generated populations of greater than 60% insulin-expressing cells that secrete insulin in response to glucose and are capable of reversing diabetes in rodents.

48

Nanofiber-Hydrogel Composite Microparticles As A Stem Cell Delivery and Pro-Regenerative Material for Soft Tissue Defects

C. Chang[1], J. Chua[2], R. Martin[3], K. Colbert[3], HQ. Mao[2,3]

[1]Department of Biomedical Engineering [2]Department of Materials Science and Engineering, [3]LifeSprout, Johns Hopkins University, School of Medicine, Baltimore, MD.

Soft tissue defects from trauma, cancer, developmental defects, and aging affects millions of patients a year, leading to disability, chronic pain, and physical defects. Therefore, there is a need to improve natural soft tissue regeneration and to improve the integration of newly generated tissue with the host environment. Currently, fat grafting and free tissue transfer are the primary approaches to repairing soft tissue defects. However, due to the invasiveness, costs, and complications arising from surgical procedures, there is a need for an alternative approach that mimics the natural healing process of fat, but also is convenient and accessible for clinicians. The nanofiber-hydrogel composite (NHC) material using a combination of hyaluronic acid and polycaprolactone nanofibers has been formulate to match the stiffness of native soft tissue, while allowing for adequate porosity for host tissue infiltration. Previous animal studies using NHC as a bulk gel has yielded improved host tissue regeneration and angiogenesis into injected site. However, as volume of the injection increased, the extent of regenerated tissue became limited. Therefore, a new formulation of NHC, through particulating the material to microparticles (~250□m) was produced. The microparticle NHC formulation was able to be loaded with hMSCs, while proliferating cells were able to penetrate the core of the particles. By increasing the surface area of hMSCs to interact with the microparticle NHCs, the hMSC-NHC combination can be used as an injectable material to improved rate of soft-tissue remodeling.

49

Elevated Levels of Glucosylsphingosine Deregulate Lysosomal Compartment in Neuronopathic Gaucher Disease

M.P. Srikanth[1], O. Awad[1], R.A. Feldman[1]

[1]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Downregulation of β-glucocerebrosidase (GCase) is a frequent event in inherited and idiopathic neurodegenerative diseases. GCase is encoded by GBA1 and catalyzes the hydrolysis of glucosylceramide (GluCer), the first step in the biosynthesis of glycosphingolipids (GSLs). Mutations in enzymes of sphingolipid metabolism are the cause of >70 lysosomal storage disorders. Most sphingolipidoses cause neurodegeneration, indicating that sphingolipid balance is essential for neuronal survival. Severe bi-allelic mutations in GBA1 cause fatal types 2 and 3 neuronopathic Gaucher disease (nGD), while mono-allelic GBA1 mutations are the highest known risk factor for Parkinson's disease (PD). Brains from patients with nGD have 1000-fold elevated levels of glucosylsphingosine (GluSph), a neurotoxic metabolite of GluCer and key biomarker of GD. To investigate the impact of sphingolipid imbalance in neuronal development, we generated neural progenitor cells (NPCs) from nGD and WT iPSCs. nGD NPCs had elevated levels of GluCer and GluSph. Further analysis showed that GCase deficiency caused lysosomal depletion in nGD NPCs and differentiated neurons. These lysosomal abnormalities were prevented by incubation with recombinant GCase. To directly test the hypothesis that GluSph is responsible for the lysosomal abnormalities of nGD, we incubated WT NPCs with exogenous GluSph. Immunofluorescence analysis showed that treatment of WT-NPCs with GluSph caused widespread lysosomal depletion as determined by decreased expression of the lysosomal markers LAMP1 and LAMP2, as well as lysotracker staining. Thus, GluSph treatment phenocopied the lysosomal depletion observed in nGD NPCs. Lastly, we examined the effects of glucosylceramide synthase inhibitors used in substrate reduction therapy, an FDA-approved treatment for type 1 GD that prevents the build-up of GSLs. This treatment restored lysosomal homeostasis in nGD NPCs. We are currently working to identify the molecular sensors of sphingolipid imbalance that are responsible for lysosomal dysfunction and neurodegeneration, and candidate molecules will be described. We are also using this system to evaluate the therapeutic efficacy of glucosylceramide synthase inhibitors for reversing neurodegeneration in nGD.

50

The DNA Methylation and Regulatory Elements During Chicken Germ Stem Cell Differentiation

Yanghua He, Jiuzhou Song

Department of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742, USA

The production of germ cells in vitro would open important new avenues for human medicine, but the mechanisms of germ cell differentiation are not well understood. The aim of our study was to use chicken as a model to explore its regulatory mechanisms. We reported a comprehensive atlas at the genome-wide DNA methylation landscape in chicken germ stem cells and transcriptomic dynamics was also presented. By uncovering DNA methylation patterns on single genes, some genes accurately modulated by DNA methylation were found to be associated with cancers, leukemia, and virus infection e.g. AKT1, CCND1, MYC, CTNNB1, and PTEN. Chicken unique markers were first discovered for identifying male germ stem cells compared to the studies of human and mouse. Moreover, the integrated epigenetic mechanisms were explored during chicken male germ cell differentiation, which provides evidence to understand the epigenetic processes associated with male germ cell differentiation in animals and humans. Collectively, our combined findings demonstrate that germ cell differentiation is not only accompanied by an organized DNA methylation changes but also regulated by multifaceted factors including transcriptional regulator complexes and histone modifications, which are similar across species and help us unveil the mechanisms in modulating gene expression during germ cell differentiation.

51

Building A Commercial Path for EpiX™ technology: A Breakthrough in Expanding and Utilizing Tissue-Resident Stem Cells

Chengkang (CK) Zhang[1], Anura Shrivastava[1], Hermann Bihler[2], Jan Harrington[2], Isaac Musisi[2], Martin Mense[2], Scott H. Randell[3], Matthew J. Bruehl[4], Marianne S. Muhlebach[4], Travis J. McQuiston[1], Sherry S. Challberg[1] and Brian A. Pollok[1]

[1]Propagenix Inc., 9605 Medical Center Drive, Suite 325, Rockville, MD 20875
[2]Cystic Fibrosis Foundation, 44 Hartwell Avenue, Lexington, MA 02421
[3]Marsico Lung Institute, UNC School of Medicine, Chapel Hill, NC 27599
[4]Department of Pediatrics, UNC School of Medicine, Chapel Hill, NC 27599

With the support of MSCRF commercialization grant, we successfully built the commercial path for the EpiX™ technology – a breakthrough in culturing tissue-resident epithelial stem and progenitor cells from diverse tissues for studying epithelial cell biology in vitro and unleashing their potential for regenerative cell therapy. In this project, our work aimed at 1) illustrating the unparalleled competitive advantage of EpiX technology over conventional methods using human airway epithelial cells as an example; 2) demonstrating the utility of EpiX technology for personalized medicine applications by expanding nasal epithelial cells out of minimal-invasive nasal brushing samples obtained from cystic fibrosis patients; and 3) scaling up EpiX medium production from bench scale to lot size of 100 liters, to provide support for moving forward with full commercialization. Summary: In AIM 1, we verified that the EpiX medium supported the expansion of primary airway epithelial cells from two different donors for over 50 population doublings, while retaining consistent functionality as assessed by various molecular and cellular characterization assays by the end of 1Q 2018. This is a significant improvement over current practice, and allows researchers to generate billion-fold more airway epithelial cells for their research study than using the convention methods. In AIM 2, we tested a personalized medicine approach for cystic fibrosis patients by using patient-derived nasal epithelial cells for functional in vitro drug response evaluation assay. We successfully generated 50–100 millions of nasal epithelial cells using brushing samples from 7 adolescent CF patients (4 males and 3 females, 7/7, 100% success), and tested their responses to Ivacaftor and Lumacaftor using the Ussing chamber assay, a “gold standard” drug efficacy evaluation assay for CFTR-directed therapeutics. The results revealed distinct responses towards the same drug despite that these patients share the same CFTR mutation. In AIM 3, we have successfully established the production SOPs and QC specifications for the EpiX medium, including analytical and functional quality tests and specifications such as standard pH, sterility, osmolality, endotoxin level and support of cell growth and differentiation. So far, we have manufactured 4 lots of EpiX medium at a scale of 100 liters and delivered high-quality, reproducible products for many researchers working with airway epithelial cells, including international customers. License of EpiX technology: STEMCELL Technologies, a leader of research tool company dedicated in supporting the stem cell research community, has signed an exclusive license agreement with Propagenix to commercialize EpiX technology and bring this revolutionary product to the research community through its distribution network.Grant-related publications: One poster presented at 2018 ATS conference, and one manuscript submitted to Cell Reports (in revision) Patents: Three US patents (9,790,471, 9,963,680 and 10,066,201) on the EpiX™ technology have been granted.

52

NF-KB p50 Deficient Immature Myeloid Cells (p50-IMC) As A Novel Cancer Immunotherapy

Rahul Suresh, Theresa Barberi, David J. Barakat, Kenneth J. Pienta, and Alan D. Friedman

Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Multiple solid tumors, including melanoma, fibrosarcoma, colon, prostate, and pancreatic carcinoma, glioblastoma, and neuroblastoma exhibit impaired growth in p50-/- mice lacking the inhibitory NF-kB p50 subunit, associated with activated tumor-associated macrophages and T cells. We hypothesize that these effects result from intrinsic activation of tumor myeloid cells, with consequent tumor-infiltrating T cell activation, and envision a cell-based therapy wherein patient-derived myeloid progenitors are expanded while targeting their p50 alleles and then infused back into patients. To validate this therapeutic approach, mice were inoculated with Hi-Myc prostate cancer (PCa), GL261 glioblastoma (GBM), or K-Ras(G12D) pancreatic ductal carcinoma (PDC) cells, the latter two lines expressing exogenous luciferase. Once tumor growth was evident, mice received a dose of 5-fluorouracil (5FU) followed five days later by myeloid cell therapy. 5FU was provided to reduce bone marrow production of competing myeloid cells, as well as to reduce tumor myeloid cell numbers and to release tumor neoantigens to favor T cell activation. To generate immature myeloid cells (IMC) for infusion, marrow cells from p50-/- or wild-type (WT) mice were expanded in media containing SCF/TPO/FL and transferred briefly to M-CSF. The IMC were then administered intravenously every 3-4 days (1E7 cells/dose for three doses). Immature rather than mature cells were infused as these are expected to more effectively by-pass lung and liver to reach tumor and lymph nodes. Mice bearing PCa showed significantly slowed tumor growth after receiving 5FU followed by p50-IMC compared with mice receiving WT-IMC or 5FU alone (p=0.001), with 5-fold reduced tumor volume on day 35. For mice implanted with GBM, 3 of 5 manifested very small tumors on day 21, in contrast to the control groups, and 4 of 10 PDC tumors shrank >10-fold in response to 5FU followed by p50-IMC. Progeny of CD45.2+ IMC were tracked in CD45.1+ tumor-bearing hosts. p50-IMC-derived CD11b+ myeloid cells were evident in prostate tumor, draining lymph nodes, spleen, and marrow, with tumor and nodal F4/80+ macrophages displaying an activated MHCII+CD11c+ phenotype. In addition, tumor CD8 T cell numbers were 5-fold increased after p50-IMC compared with WT-IMC, and these manifested an activated phenotype, with increased interferon expression in response to PMA/ionomycin. Adoptive cell transfer of p50-IMC, following a dose of 5FU, activates tumor myeloid and T cells to slow murine tumor growth and predicts the therapeutic utility of human p50-IMC against multiple solid tumors.

53

Monitoring the Degradation of Implanted Hydrogels Using CEST MRI

Wei Zhu[1,2], Chengyan Chu[1,2], Shreyas Kuddannaya[1,2], Yue Yuan[1,2], Piotr Walczak[1,2], Anirudha Singh[3,4], Xiaolei Song[1,2], Jeff W.M. Bulte[1,2,4]

[1]Russell H. Morgan Dept. of Radiology and Radiological Science, Division of MR Research, The Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]Cellular Imaging Section, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA; [3]Dept. of Urology, The James Buchanan Brady Urological Institute, The Johns Hopkins School of Medicine, Baltimore, MD, USA; [4]Dept. of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA.

Introduction: Stem cell therapy has garnered much attention for treatment of neurodegeneration. However, the harsh microenvironment at the site of damaged tissue often impairs the retention and survival of transplanted stem cells. Scaffolding cells with hydrogels is a promising strategy to overcome initial cell loss and manipulate cell function post-transplantation. While scaffold materials are essential for improving survival of cellular grafts, matrix degradation is a major requirement for functional graft integration from implanted biomaterials. [1]. Therefore, monitoring the degradation of hydrogels is essential for developing successful scaffolded stem cell therapies. We investigated whether CEST MRI can visualize implanted hydrogel scaffold degradation in mouse brain.
Methods: A covalently cross-linked hydrogel composing of 20 mg/mL thiol-modified gelatin, 20 mg/mL thiol-modified hyaluronic acid (HA), and 20 mg/mL polyethylene (glycol) diacrylate (PEGDA) (crosslinker) was prepared. The ratio of gelatin to HA was varied in an effort to achieve the optimal formulation for CEST contrast. The amount of PEGDA equals 25% of the total volume of gelatin and HA. In vitro CEST MRI was carried out at 37 °C. For in vivo visualization, 3 µL hydrogel was injected into mouse striatum. CEST MRI was performed using a horizontal bore 11.7 T Bruker scanner till 42 days. To validate the In vivo CEST MRI findings and identify the main decomposing component in the hydrogel, gelatin and HA were labeled green and red near-infrared (NIR) dyes, respectively. The stained hydrogel was then visualized over time using a LI-COR optical in vivo imaging system. Results and Discussion: In vitro CEST MRI demonstrated a peak at 3.6 ppm for saturation field strengths from 1.2 to 7.2 µT. A value of 3.6 µT exhibited a comparable CEST signal with higher B1 values, but a less broad spectrum, and was therefore used for all further experiments. When we examined the three individual components of the hydrogel, we found that gelatin is the major contributor to the CEST signal at 3.6 ppm, in agreement with earlier studies [2]. We then increased the proportion of gelatin in the hydrogel. The increase of gelatin enhanced CEST contrast (Fig. 1a). When the hydrogel was injected into the brain of mice, it was clearly distinguished from the surrounding native tissue (Fig. 1b). This attenuation of CEST contrast indicates the feasibility of CEST MRI to monitor the degradation of hydrogel over time. The NIR signal of gelatin decrease gradually over 42 days, while the HA signal remained relatively stable (Fig. 3c). This suggests that gelatin, the main source of CEST signal at 3.6 ppm, is the main decomposing component in the hydrogel. An excellent correlation was found between the decay of CEST signal and gelatin NIR fluorescence signal (R2=0.94, Fig. 3d).
Figure 1. (a) CEST map of different hydrogels injected in the striatum obtained at 3.6 ppm for B1=3.6 µT. Ratios represent gelatin:HA. (b) T2-w CEST overlay images of 4:1 hydrogel measured at 3.6 ppm from day 1 to day 42. Arrow indicates hydrogel injection site. (c) Serial NIR optical images of the same mice shown in b and photon quantification (n=4). 800CW and 680LT correspond to labeled gelatin (green) and HA (red), respectively. (d) Correlation of in vivo CEST signal with NIR fluorescence intensity values shows an excellent correlation for gelatin (R2=0.94), but not for HA (R2=0.45, data not shown).
Conclusion: Hydrogel degradation can be visualized in a non-invasive and label-free manner. Gelatin was found to be the major contributor of CEST contrast and also the main degradation component in the hydrogel. This approach may be used further to develop hydrogels with optimal biodegradation properties for scaffolded stem cells. References: [1] Madl CM, et al. Nature Materials. 2017;16:1233. [2] Liang Y. et al. Biomaterials 2015;42:144.

54

Development of a 3D Fluorescent Imaging Technique to Observe Blood Vessel Phenotype in Tissue-Engineered Bone Grafts

Alexandra N. Rindone[1,2], and Warren L. Grayson[1,2,3,4]

[1]Dept. of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD; [2]Translational Tissue Engineering Center, Johns Hopkins University School of Medicine, Baltimore, MD; [3]Dept. of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD. [4]Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD.

Tissue engineered bone grafts (TEBGs) have demonstrated to completely heal critical-sized defects in small animal models, but their clinical success is limited due to a lack of understanding into the mechanisms of robust bone healing. In a physiological environment, specific blood vessel phenotypes – characterized by CD31/endomucin co-expression and morphology – are intimately associated with osteoprogenitors, and are necessary for proper bone growth and remodeling. However, there is minimal knowledge on how blood vessel phenotype contributes to bone formation in TEBGs. The objective of this study is to develop a 3D imaging platform for evaluating blood vessel phenotype and vessel-osteoprogenitor relationships in TEBGs. A sample preparation method was optimized to observe vessels and osteoprogenitors in 1) native murine calvaria and 2) TEBGs containing polycaprolactone (PCL)-fibrin scaffolds and human adipose-derived stem cells implanted into critical-sized murine calvarial defects. Briefly, harvested samples were fixed in 4% PFA, decalcified in 10% EDTA, and treated with chloroform to dissolve the PCL scaffolds. Then, samples were stained with antibodies and optically cleared with thiodiethanol to allow for whole-mount imaging. High resolution 3D images were generated by taking 2x2 or 3x3 tile scans on a lightsheet microscope (10x magnification, ~ 1.3 x 1.5 x 0.8 mm3 per tile, 20% x-y tile overlap), and processed using ImageJ. Our results demonstrate that this technique enabled visualization of distinct blood vessel phenotypes – characterized by CD31 and endomucin expression – in both native calvaria and in implanted TEBGs. Furthermore, we were able to visualize the distribution of Runx2-stained osteoprogenitors in native calvaria. Future work will focus on developing quantitative metrics for vessel phenotype, and elucidating the spatiotemporal relationship between blood vessel phenotypes and osteoprogenitors in TEBGs. This work will provide a better understanding of the biological mechanisms governing robust bone regeneration, and inform strategies for designing clinically-successful TEBGs.

55

Temporal Next Generation Transcriptome Screen of Human Neuronal Pro-Survival Molecules and Pathways

Xu, J.-C.*, Kang, S.-U.*, Zhu, J., Fan, J., Frazee, A., Antonescu, C., Zhou, W., Chen, L., Chi, Z., Yang, S.-Z., Ji, H., Salzberg, S., Leek, J., Dawson, T.M., Dawson, V.L.*

Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, 305 East Zhongshan Road, Nanjing, Jiangsu 210002, China; Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Alzheimer's disease and Related Dementias (AD/ADRD) and Stroke are the major devastating neurological disorders. Stroke resulting from brain infarcts, often co-exists and potentially interacts with AD. Intriguing, in both AD and stroke, significant dysregulation of N-Methyl D-Aspartic acid receptors - (NMDAR, the prime target for human cognition), as well as reduced neuronal availability of oxygen & glucose (associate with human brain evolution) occur either as cause or interactive pathologies12-15. Thus, the identification of major regulatory factors governing these 'antiquated' pathways associated with NMDAR and oxygen & glucose is interesting and imperative. The manipulation of these pathways may then provide potential targets for interventions. Extensive researches had been studied on these pathways. However, human brain tissue has inaccessible nature. The snapshot of AD and stroke through postmortem tissue has hindered the identification of dynamic changes, which may lead to neuron degeneration or survival. Models used to identify dynamic changes in human neurons need to be established. Using human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, we have developed a high-efficiency method of generating authentic human cortical neurons16, positioning us to investigate the longitudinal signaling activated. We profiled the time-series transcriptome following activation of the i) NMDAR- ii) oxygen & glucose pathway- regulated survival pathways in human cortical neurons. Transcriptomic analysis reveals that human neurons respond very similarly to either NMDAR or oxygen & glucose activation, indicating converged downstream signaling. Functional clustering analysis of the differentially expressed genes shows the enrichment of genes mediating neuroplasticity, learning & memory and neuron survival. Further upstream analysis of regulatory networks of these differentially expressed genes identified Prdm3 (MECOM/EVI1) as the one of the major regulatory factors. Intriguingly, the temporal lifespan expression pattern of Prdm3 and several other potential master regulators in human prefrontal cortex18 are very similar to the higher cognitive trajectory19,20 in human brain. This indicates Prdm3 and its mono-methyltransferase activity on histone 3 lysine 9 (H3K9me1) may regulate brain cognition and aging of brain through induction of gene expression

56

Fibrin Hydrogel Small-Diameter Graft as An Arterial Conduit

Morgan Elliott*[1,2]*, Brian Ginn[2,3,4], Takuma Fukunishi[5], Djahida Bedja[6], Abhilash Suresh[1,2], Theresa Chen[2,7], Harry C. Dietz[6,8,9], Lakshmi Santhanam[10], Hai-Quan Mao[1,2,3,4], Narutoshi Hibino[2,5], Sharon Gerecht[2,3, 7,11]*

[1]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2]Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD, USA; [3]Department of Materials Science and Engineering, Johns Hopkins University Whiting School of Engineering, Baltimore, MD, USA; [4]Translational Tissue Engineering Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [5]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [6]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [7]Department of Chemical and Biomolecular Engineering, Johns Hopkins University Whiting School of Engineering, Baltimore, MD, USA; [8]Department of Pediatrics and Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [9]Howard Hughes Medical Institute, Chevy Chase, MD, USA; [10]Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [11]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Autologous and synthetic polymer small-diameter grafts used for arterial bypass surgery are insufficient and often lead to morbidity and mortality. Here, we report a small diameter tissue-engineered vascular graft (sTEVG) prepared from a natural polymer for arterial bypass surgery. Using a hollow fibrin microfiber tube with controlled surface topography, we enable immediate, controlled perfusion and formation of an aligned, confluent endothelium both in vitro and in vivo. Implantation of either acellular or endothelialized grafts (1.09 ± 0.17 mm in diameter) in mouse abdominal aorta supported normal blood flow and vessel patency for 24 weeks, indicating no change in vascular function due to sTEVG implantation. Acellular grafts developed clots on the luminal walls by week one in 1 of 5 of cases, but no evidence of these clots was visible in either graft type at later time points. Endothelialized grafts evidenced increased overall cell concentration, including macrophages, smooth muscle cells (SMCs), and endothelial cells at week 1 compared to acellular sTEVGs. For both acellular and endothelialized grafts, SMC penetration with collagen and elastin deposition was apparent by week 4 and increased over time. The medial layer was significantly thicker at week 8 and calcification was reduced in week 8 and 24 endothelialized grafts relative to acellular grafts, indicating enhanced remodeling. The tunica media, composed of collagen, elastin, and SMCs, became more organized into layers, similar to the banding seen in the native aorta by week 24. After implantation, the ultimate circumferential stress and strain and the elasticity of the acellular and endothelialized grafts closely approximated the native abdominal aorta. Additionally, the elasticity of the week-24 sTEVGs closely approximated the native control. Overall, we show that our sTEVG composed of a natural polymer matches the native vessel size and mechanical properties, incorporates into the native vascular tissue, has low thrombogenicity, and exhibits a clinically relevant shelf life; thus, it warrants further investigation towards arterial bypass surgery clinical trials.

57

Non-Invasive Imaging of Hydrogel Scaffold Biodegradation And Transplanted Cell Survival

Wei Zhu, Kang, Shreyas Kuddannaya, Chengyan Chu, Yue Yuan, Piotr Walczak, Anirudha Singh, Xiaolei Song, Jeff Bulte

Russell H. Morgan Dept. of Radiology and Radiological Science and Cellular Imaging Section, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

INTRODUCTION: Stem cell therapy has garnered much attention for treatment of neurodegeneration. However, the harsh microenvironment at the site of damaged tissue often impairs the retention and survival of transplanted cells. Scaffolding cells with hydrogels is a promising strategy to overcome initial cell loss and manipulate cell function post-transplantation. While scaffold materials are essential for improving survival of cellular grafts, matrix degradation is a major requirement for functional graft integration from implanted biomaterials [1]. Therefore, monitoring the degradation of hydrogels is essential for developing successful scaffolded stem cell therapies. It is also essential to study the survival of transplanted stem cells to eventually promote integration and differentiation in the host tissue. We investigated whether CEST MRI can visualize implanted hydrogel scaffold degradation in mouse brain, and used bioluminescence imaging (BLI) to probe transplanted cell survival. METHODS: A covalently cross-linked hydrogel composing of 20 mg/mL thiol-modified gelatin, 20 mg/mL thiol-modified hyaluronic acid (HA), and 20 mg/mL polyethylene (glycol) diacrylate (PEGDA) (crosslinker) was prepared. In vitro CEST MRI was carried out at 37 °C. For in vivo visualization, 3 µL hydrogel was injected into mouse striatum. CEST MRI was performed over 42 days using a horizontal bore 11.7 T Bruker scanner. To validate the in vivo CEST MRI findings and identify the main decomposing component in the hydrogel, gelatin and HA were labeled with green and red near-infrared (NIR) dyes, respectively. The labeled hydrogel was then visualized over time using a LI-COR optical in vivo imaging system. Additionally, mouse glial restricted progenitor cells (mGRPs) expressing green fluorescent protein (GFP) and transduced with a lentiviral vector carrying firefly luciferase (pLenti4-CMV-Luc) were encapsulated within the hydrogels. The survival of the transplanted cells with/without scaffolding (hydrogel) was monitored with a Perkin Elmer IVIS Spectrum/CT instrument. RESULTS AND DISCUSSION: In vitro CEST MRI demonstrated a peak at 3.6 ppm for saturation field strengths from 1.2 to 7.2 µT. A value of 3.6 µT exhibited a comparable CEST signal with higher B1 values, but a less broad spectrum, and was therefore used for all further experiments. When we examined the three individual components of the hydrogel, we found that gelatin is the major contributor to the CEST signal at 3.6 ppm, in agreement with earlier studies [2]. When the hydrogel was injected into the brain of mice, it could be clearly distinguished from the surrounding native tissue. This attenuation of CEST contrast indicates the feasibility of CEST MRI to monitor the degradation of hydrogel over time. The NIR signal of gelatin decrease gradually over 42 days, while the HA signal remained relatively stable. This suggests that gelatin, the main source of CEST signal at 3.6 ppm, is the main decomposing component in the hydrogel. An excellent correlation was found between the decay of CEST signal and gelatin NIR fluorescence signal (R2=0.94). When mGRPs were injected into brain, scaffolded mGRPs showed prolonged in vivo survival and restricted localization at the injection site relative to unscaffolded mGRPs. CONCLUSIONS: Hydrogel degradation can be visualized in a non-invasive and label-free manner. Gelatin was found to be the major contributor of CEST contrast and also the main degradation component in the hydrogel. Scaffolding of GRPs in biocompatible and mechanically stable hydrogels can confer higher graft stability with minimal cell loss, leading to prolonged cell survival. Our dual-mode imaging approach may be used to develop hydrogels with optimal biodegradation properties for scaffolded stem cells. REFERENCES: [1] Madl CM, et al. Nature Materials. 2017;16:1233. [2] Liang Y. et al. Biomaterials 2015;42:144. ACKNOWLEDGEMENTS: This study was supported by MSCRFD-3899.

58

Optimal Electrical Stimulation Boosts Nerve Regeneration with Stem Cell Therapy

Jian Du[1], Gehua Zhen[2], Huanwen Chen[1], Shuming Zhang[3,4], Liming Qing[1], Xiuli Yang[1], Gabsang Lee[5,6], Hai-Quan Mao[3,4], Xiaofeng Jia[1,3,7,8,9]*

Department of [1]Neurosurgery, [7]Orthopedics, [8]Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201, Dept. of [2]Orthopaedics, [3]Biomedical Engineering, [4]Materials Science and Engineering, [4]Neurology, 5Neuroscience, [9]Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Peripheral nerve injuries often lead to incomplete recovery and contribute to significant disability to approximately 360,000 people in the USA each year. Stem cell therapy holds significant promise for peripheral nerve regeneration, but maintenance of stem cell viability and differentiation potential in vivo are still major obstacles for translation. Here we investigated the effects of different electrical stimulating parameters on human neural crest stem cells (NCSCs) differentiation in vitro, and then evaluated the effect of optimized electrical stimulation (ES) on human neural crest stem cell (NCSC) transplantation for peripheral nerve regeneration.15 mm critical-sized sciatic nerve injuries were generated with subsequent surgical repair in sixty athymic nude rats. Injured animals were randomly assigned into five groups (N=12 per group): blank control, ES, NCSC, NCSC+ES, and autologous nerve graft. The optimized ES from the in vitro studies was applied immediately after surgical repair for 1h in ES and NCSC+ES groups. Recovery was assessed by behavioral (CatWalk gait analysis), wet muscle-mass, histomorphometric, and immunohistochemical analyses at either 6 or 12 weeks after surgery (N=6 per group). Gastrocnemius muscle wet mass measurements in ES+NCSC group were comparable to autologous nerve transplantation and significantly higher than other groups ($p<0.05$). Quantitative histomorphometric analysis and catwalk gait analysis showed similar improvements by ES on NCSCs ($p<0.05$). A higher number of viable NCSCs was shown via immunochemical analysis, with higher Schwann cells (SC) differentiation in the NCSC+ES group compared to the NCSC group ($p<0.05$). Overall, ES on NCSC transplantation significantly enhanced nerve regeneration after injury and repair, and was comparable to autograft treatment. Thus, ES can be a potent alternative to biochemical and physical cues for modulating stem cell survival and differentiation. This novel cell-based intervention presents an effective and safe approach for better outcomes after peripheral nerve repair.

Acknowledgments
Supported by Maryland Stem Cell Research Fund

59

Induced Pluripotent Stem Cell Derived Endothelial Cells Display Unique Phenotype in Low Oxygen Environments

Bria Macklin[1], Sharon Gerecht[1,2]

[1]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21208, [2]Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21218, United States

Objective: Hypoxia, or low oxygen tension (<5%), is a condition that has been shown to govern vasculogenesis as the embryo develops and the need for oxygen increases in newly formed tissues. Here, we aim to study how hypoxia regulates vascular network formation of human induced pluripotent stem cell (hiPSCs) derived endothelial cells (ECs). Methods: Using our bi-potent differentiation protocol, we generate early vascular cells (EVCs) that consist of PDGFRβ+/SM22α+ early pericytes and VECad+/CD31+ early ECs from hiPSCs, which can be further matured into functional vascular cells. VECad+/CD31 ECs or HUVECs are encapsulated in collagen hydrogels, stimulated with VEGF and allowed to form vascular networks over 3 days. “Hypoxic hydrogels” are generated by balancing O2 diffusion with O2 cell consumption. Oxygen saturation in hydrogels is measured using non-invasive fluorescence quenching O2 sensors. Results: HUVECs encapsulated in the “hypoxic hydrogels” reached O2 saturation ~1% and generated vascular networks similar to those in the nonhypoxic hydrogels. In contrast, hiPSC-ECs encapsulated in “hypoxic hydrogels” formed larger and thicker tubes with overall increased number of tubes as compared to nonhypoxic hydrogels, but did not reach below 10% O2 saturation. Treatment with a ROS inhibitor, diphenyleneiodonium (DPI), led to similar network inhibition in both mature and hiPSC-ECs. Examining the oxygen uptake rate we found an adaptation of the hiPSC-EC to hypoxia but not in HUVECs. Current studies focus on delineating the underlying mechanism.

60

Stem Cells to Reprogram Skin Identity and Enhance Prosthetic Use in Amputees

Luis Garza, MD-PhD, Johns Hopkins University School of Medicine

Background: Amputation rates in recent conflicts have been high. Although major advancements have been made in prosthetics, their use is still limited by the skin at the stump site. Most notably discomfort and pain, but also skin breakdown result because the skin flap at the stump sites was not adapted to bear weight. We propose to reprogram the skin at the stump site to be the thick skin at the palms and soles (volar skin) to make it more weight-, friction- and irritant-resistant. Methods: Under grants from MSCRF we are currently performing a cellular therapy clinical trial on human subjects to create ectopic volar skin. We are injecting autologous volar fibroblasts to non-volar areas and measuring for changes towards volar skin. We are also testing in vitro what factors control reprograming of non-volar epithelial cells to volar epithelial cells. Results: We have full regulatory approval and have enrolled more than 15 subjects testing a variety of variables. Using genome-wide analysis, we show that KERATIN 9 (KRT9) is the most uniquely enriched transcript in volar keratinocytes. KRT9 expression in keratinocytes is upregulated by volar compared to non-volar fibroblast in both mouse models and in vitro co-cultures. In our Phase 1 clinical trial, KRT9 protein expression is selectively upregulated by volar compared to nonvolar fibroblasts. Additionally, we find that cytoplasmic size—notably higher in native volar keratinocytes-- also increases in non-volar keratinocytes exposed to volar fibroblasts. These results suggest this cellular therapy is modifying skin identity both in gene expression and in overall architecture. Conclusions: Cellular therapy is a promising avenue to reprogram skin identity in amputees at the stump site to become thick palmo-plantar skin in an effort to enhance prosthetic use. Planning for a phase 2 trial in amputees is currently in progress.

61

NF-KAPPAB Pathway Is Involved in Bone Marrow Stromal Cell-Produced Pain Relief

*Wei Guo[1], Satoshi Imai[1,2], Jia-Le Yang[1], Shiping Zou[1], Huijuan Li[1,3], Huakun Xu[4], Kamal D. Moudgil[5], Ronald Dubner[1], Feng Wei[1], Ke Ren[1]**

[1]Dept. of Neural & Pain Sciences, School of Dentistry, & Program in Neuroscience, Univ. of Maryland, Baltimore, MD; [2]Present Affiliation: Dept. of Clinical Pharmacology & Therapeutics, Kyoto Univ. Hospital, Kyoto, Japan; [3]Dept. of Neurology, The 3rd Affiliated Hospital of Sun Yat-sen Univ., Guangdong Province, China; [4]Dept. of Advanced Oral Sciences and Therapeutics, Univ. of Maryland School of Dentistry, Baltimore, MD ; [5]Dept. of Microbiology & Immunology, Univ. of Maryland, Baltimore, MD

Bone marrow stromal cells (BMSCs) produce long-lasting attenuation of pain hypersensitivity. This effect involves BMSC’s ability to interact with the immune system and activation of the endogenous opioid receptors in the pain modulatory circuitry. The nuclear factor kappa B (NF-ϰB) protein complex is a key transcription factor that regulates gene expression involved in immunity. We tested the hypothesis that the NF-ϰB signaling plays a role in BMSC-induced pain relief. We focused on the rostral ventromedial medulla (RVM), a key structure in the descending pain modulatory pathway, that has been shown to play an important role in BMSC-produced antihyperalgesia. In Sprague Dawley rats with a ligation injury of the masseter muscle tendon (TL), BMSCs (1.5M/rat) from donor rats were infused i.v. at 1 w post-TL. P65 exhibited predominant neuronal localization in the RVM with scattered distribution in glial cells. At 1 w, but not 8 w after BMSC infusion, western blot and immunostaining showed that p65 of NF-ϰB was significantly increased in the RVM. Given that chemokine signaling is critical to BMSCs’ pain-relieving effect, we further evaluated a role of chemokine signaling in p65 upregulation. Prior to infusion of BMSCs, we transduced BMSCs with Ccl4 shRNA, incubated BMSCs with RS 102895, a CCR2b antagonist, or maraviroc, a CCR5 antagonist. The antagonism of chemokines significantly reduced BMSC-induced upregulation of p65, suggesting that upregulation of p65 was related to BMSCs’ pain-relieving effect. We then tested the effect of a selective NF-ϰB activation inhibitor, BAY 11-7082. The mechanical hyperalgesia of the rat was assessed with the von Frey method. In the pre-treatment experiment, BAY 11-7082 (2.5 and 25 pmol) was injected into the RVM at 2 h prior to BMSC infusion. Pretreatment with BAY 11-7082 attenuated BMSCs’ antihyperalgesia, but post-treatment at 5 w post-BMSC was not effective. On the contrary, in TL rats receiving BAY 11-7082 without BMSCs, TL-induced hyperalgesia was attenuated, consistent with dual roles of NF-κB in pain hypersensitivity and BMSC-produced pain relief. These results indicate that the NF-ϰB signaling pathway in the descending circuitry is involved in initiation of BMSC-produced behavioural antihyperalgesia. Supported by the MSCRF and NIH; HL is supported by The Team-Building Project for Stem Cell Research, SYSU.

62

Clinical Scale Production and Wound Healing Activity of Human Adipose Derived Mesenchymal Stem Cell Extracellular Vesicles from a Hollow Fiber Bioreactor

John J.S. Cadwell, FiberCell Systems Inc. and John Ludlow, Zen Bio

Introduction: Production of extracellular vesicles (EV) such as exosomes at the scale required for clinical applications remains a challenge. Current methods can utilize large numbers of flasks and serum starvation in a batch mode process. Hollow fiber bioreactors are perhaps ideal for producing large quantities of EV at 100X higher concentrations than conventional protocols. Hollow fiber bioreactors support the culture of large numbers of cells at high densities, 1-2X10e8 cells/ml. Cells are bound to a porous support with a 20kDa molecular weight cut off (MWCO) so cell passaging is not required and EV cannot cross the fiber in either direction. Methods: 1X109 adipose derived adult MSC were cultured in a FiberCell hollow fiber bioreactor for 8 weeks in DMEM+10% FBS in the circulating medium only. Cells did not expand (monitored by glucose uptake rate) nor did they differentiate (by multiple immunocytochemistry assays) over this time. 40 ml of conditioned medium from the extra-capillary space was harvested weekly. A scratch closure test and rat skin wound healing assay was used to measure wound healing activity. Results: 8.6X1011 EV particles in a volume of 120mls were harvested from the adult adipose derived MSC culture. 18 liters of DMEM were consumed over 8 weeks. Additionally, EV/protein was 10-fold higher in harvests from the bioreactor suggesting higher purity as well. Wound healing assays demonstrated significant acceleration of wound healing. Summary/conclusion: Hollow fiber bioreactors have demonstrated potential for the manufacturing scale production of EV using cGMP compliant materials and methods. The EV isolated from adult adipose derived MSC cultured in an HFBR show activity that can promote wound healing both in in vitro and in vivo assays. Hollow fiber bioreactors provide a number of significant advantages compared to flask based protocols including higher concentrations, large capacity, time and space efficiency, and perhaps EV quality. Current available technology permits the production of gram quantities of EV, with potential use for clinical applications.

63

Agriculture Is No Longer Beverly Hillbillies or Green Acres

Joyce Hunter, Vulcan Enterprises, LLC.

Triggering students' interest in pursuing more technical fields begins in schools. There are many effective strategies for engaging students and improving their performance in STEAM subjects which makes the business case for the Open Data Science Technology Engineering Agriculture and Math (STEAM) Summer Camp Program compelling. One vital benefit of open data, making information resources easy to find, accessible, and usable can fuel entrepreneurship, innovation, and scientific discovery that improves Americans' lives and contributes significantly to job creation. Open Data has become both a tactical and strategic role in organization's operations and policies and will require personnel to fulfill emerging skill-sets such as data analysts, quality assurance specialists and data officers. Organizations that have Open Data as part of their strategic workforce planning are able to anticipate future needs and utilize lead time to foster the development of new skills internally before they become mission critical. They need to build the capacity to think as a single enterprise about its IT workforce development and we believe we can start that process with the Open Data STEAM Summer Camp program. We must adapt our IT workforce to continue to deliver value, primarily by recognizing information as a strategic asset and by creating an information-focused IT organization. Several new kinds of information professionals will staff this organization (e.g. data scientists, information managers, information architect, information leader, digital archivists, data and information visualization designers, data/information stewards). Roles will also be needed to govern, manage, and analyze open and big data . The skills required to perform these roles generally do not exist at in abundance today, which will result in a shortfall for these positions. We need to prepare for this evolution and culture shift today. The Open Data STEAM Summer Camp program will enable organizations to: 1) link workforce requirements directly to missions and aid in data collection; 2) develop a comprehensive picture of the data through a process of cataloging and analysis; 3) identify and implement data quality strategies, especially for priority data sets; and 4) identify and overcome internal and external barriers to accomplishing strategic data identification goals. Vulcan Enterprises, LLC. proposes a roadmap for the Open Data STEAM Summer Camp that would establish a continuous cycle between your organization and an academic institution, industry or government.This lifecycle would be comprised of seven basic steps: #1. Proposal presentation and agreement; #2. Communication Plan development and delivery; #3. Application development and delivery; #4. Applicant acceptance; #5. Camp implementation; #6. Evaluate and Measure (End of summer); #7. Lessons Learned / Adjust Plan as Necessary for next year.

64

Development of an iPSC Derived Cellular Model of Barth Syndrome

Arianna Franca Anzmann[1], Steven M. Claypool[2], Hilary J. Vernon[1,3]

[1]McKusick-Nathans Institute of Genetic Medicine, [2]Dept. of Physiology, [3]Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore MD.

Barth Syndrome (BTHS, 3-methylglutaconic aciduria type II, MIM #302060) is an X-linked inborn error of mitochondrial phospholipid metabolism, caused by pathogenic variants in the gene tafazzin (TAZ). TAZ encodes for a transacylase involved in the final remodeling step of cardiolipin (CL), a phospholipid localized to the inner mitochondrial membrane with key roles in cristae formation, organization of the mitochondrial respiratory chain, and in the apoptotic cascade. Deficiency of TAZ results in abnormal CL content, including an accumulation of monolysocardiolipin (MLCL) and a reduction of remodeled CL. As is typical of most primary mitochondrial diseases, BTHS is a multisystem disorder and is characterized by cardiomyopathy, skeletal myopathy, and neutropenia, among other features. However, in sharp contrast to many mitochondrial diseases, BTHS has minimal neurological burden. It is not known why TAZ, an ubiquitously expressed protein, leads to the unique tissue distribution of BTHS. In order to explore novel areas of cellular dysfunction, we applied a multi-omics discovery approach to a CRISPR-edited TAZ-deficient HEK293 cell line that was developed and phenotypically validated in our laboratory. With this approach, we discovered differential expression of proteins involved in dynamic responses to mitochondrial stress (i.e. machinery of fission, fusion, and mitophagy) and bioenergetic responses to mitochondrial stress (i.e. downregulation of ROS production).

With these findings in hand, we next set out to determine whether the dynamic and bioenergetic responses to mitochondrial stress are more dysregulated in cell types that are clinically affected in BTHS. In order to accomplish this, we developed a novel cellular model of TAZ deficiency in iPSCs. CRISPR/Cas9-gRNA was used to edit the iPSCs cells within exon 2 of TAZ. A resultant 50bp deletion was identified in 4 individually isolated clones. The frameshift deletion results in complete loss of TAZ. Further studies in this novel model, with differentiation to cardiomyocytes and neurons, has the potential to provide insight into the role of CL composition in mitochondrial function, as well as to offer novel treatment targets for BTHS.

65

Mechanical Characterization of Engineered Cardiac Tissues for Clinical Application

Seungman Park[1], *Cecillia Lui*[2], *Wei-Hung Jung*[1], *Debonil Maity*[1], *Chin Siang Ong*[2], *Joshua Bush*[3], *Venkat Maruthamuthu*[3], *Narutoshi Hibino*[2,5], *Yun Chen*[1,4,5]*

[1]Department of Mechanical Engineering, Johns Hopkins University, MD, USA [2]Division of Cardiac Surgery, Johns Hopkins University, MD, USA; [3]Department of Mechanical and Aerospace Engineering, Old Dominion University, VA, USA; [4]Center for Cell Dynamics, Johns Hopkins University, MD, USA; [5]Institute for NanoBio Technology, Johns Hopkins University, MD, USA

Heart failures (HFs) is the leading cause of death in the U.S. Currently, the most effective way to treat HF is organ transplantation. However, this method has limitations due to the lack of organ donors, immune response to the transplanted organ, and high costs. Recently, tissue engineering approaches are being widely explored to address the challenges. Among those, cardiac patch is a promising replacement for the damaged tissues. Acellular patches consisting of ECM proteins were shown to effectively resuscitate injured myocardium in animal models. In addition, 3D bioprinting, where cells and ECM proteins are organized in specific geometry and dimensions to yield functional cardiac patch warrants a potential solution to treat HFs. Nonetheless, a major obstacle described in the following remains to be surmounted before engineered tissues can be used as a general treatment option. Direct mapping of mechanical force and stress over 3D cardiac tissues is yet to be developed, as opposed to its 2D counterparts, despite that 3D cardiac tissues are more physiologically representative and can be readily applied clinically. In this study, as a proof of concept, 3D bioprinted cardiac tissues derived from human induced pluripotent stem cells (hiPSCs) are tested. First, the engineered cardiac tissues labeled with particles are recorded and tracked to determine spatially and temporally variable contraction forces. Viscoelastic properties are measured using magnetic tweezers. The results are used to compute 3D force and stress distribution over the engineered tissue by finite element method. In summary, a framework is developed to assess clinical-grade engineered cardiac tissues and determine the appropriate value ranges suitable for implantation. The results relating contractility, intrinsic mechanical properties and stress distribution in the engineered tissue, can also inform a better design for future fabrication of engineered tissues.

66

Mechanisms of Angiogenesis Within Bioprinted Endothelial: Mesenchymal Stem Cell Cocultures

Marco Santoro, *Tolulope Awosika, Kirstie L. Coombs, John P. Fisher*

Fischell Department of Bioengineering, University of Maryland College Park, College Park, Maryland; Center for Engineering Complex Tissues, University of Maryland College Park, College Park, Maryland

Introduction: Bioprinting holds great promise in the fabrication of vascularized scaffolds, a central challenge in tissue engineering. A common strategy to fabricate such vasculature relies on the deposition of endothelial cells (ECs) and mesenchymal stem cells (MSCs). However, studies have reported contrasting results on the role of MSCs in arteriogenesis and angiogenesis. Therefore, in this study we investigated how the combination of EC:MSC drives the formation of new vasculature within bioprinted scaffolds by regulating key signaling pathways. Materials and Methods: Human umbilical vein endothelial cells (HUVECs) and rat or human MSCs (rMSCs or hMSCs, respectively) were encapsulated in gelatin methacrylate (GelMA) in different coculture ratios (1:0, 3:1, 1:1, 1:3, and 0:1) and cultured for 2 weeks. Cell-laden GelMA was extruded as fibers (400μm diameter) and evaluated for cell viability (Live/Dead) and expression of angiogenic markers (CD34, VE-cadherin, α-SMA) via fluorescent microscopy. Gene expression was analyzed via rt-PCR against VEGF, PDGF-BB, TIE-2 and TGF-β1-related signaling pathways. Expression of VEGF, TGF-β1, PDGF-BB, and Ang-1 ligands was measured via ELISA. All data were analyzed using a one-way ANOVA test ($p < 0.05$), followed by a post hoc Tukey's HSD test. Results and Discussion: Cell-laden GelMA scaffolds were successfully bioprinted with no detrimental effects on cell viability. Gene expression showed upregulation of angiogenic markers in the EC:MSC 3:1 group compared to homotypic cultures. Interestingly, we observed a physiologically relevant pattern with initial upregulation of angiogenesis in ECs (VEGF, PDGF) followed by enhanced arteriogenesis in MSCs (PDGF, TGF-β1) within hybrid HUVEC/rMSC cocultures at later time points. These findings were corroborated in fully human HUVEC/hMSC cocultures, where hMSC exert an antiangiogenic effect early in the culture and a proangiogenic effect after 7 days in coculture. Conclusions: In bioprinted EC:MSC cocultures, early angiogenesis is driven by ECs, while later arteriogenesis is mainly regulated by MSCs. During this process, EC:MSC crosstalk synergistically promotes up-regulation of angiogenic and arteriogenic pathways in both cells. In future studies, this basic knowledge will serve as the design criteria to fabricate vascularized scaffolds of a clinically relevant size.

67

A Duchenne Muscular Dystrophy Patient hiPSC-Derived Myoblasts Based Drug Screen Uncovers Potential Compounds for DMD Treatment

Congshan Sun, *Inyong Choi, Gabsang Lee, Kathryn Wagner*

Duchenne muscular dystrophy (DMD), the most common muscular dystrophy, is characterized by progressive muscle degeneration and weakness resulting in the loss of ambulation during adolescence and ultimately death in early adulthood. Currently there are only two FDA approved drugs targeting DMD, and there is a great need of more treatment options for this devastating disease.

However, due to a lack of efficient DMD disease model, drug discovery and development have been slow. Human induced pluripotent stem cell (hiPSC) which overcomes the interspecies difference of animal models and limited expandability of primary cell lines makes the large-scale drug discovery possible. Our laboratory has developed a novel serum-free hiPSC-to-skeletal muscle lineage differentiation protocol. We also characterized one of DMD patient hiPSC-derived myoblast related phenotypes as fusion defect comparing multiple lines of DMD patient human induced pluripotent stem cell (hiPSC)-derived myoblasts, which was used as a phenotype to screen compounds for DMD treatment. We performed a tiered medium-scale high-throughput chemical compound screen, composed of 1524 compounds from Johns Hopkins Clinical Compound Library, on myoblasts derived from one DMD patient specific hiPSC line. Myotube formation and associated protein expression were used as parameters to select hit compounds. We selected primary hit compounds from the primary screening followed by secondary screening to validate and optimize the concentration of positive hit compounds. Two final hit compounds were selected from the in vitro screening, followed by testing in MDX mice model. Currently we are also working on the action mechanisms of the selected drug using unbiased transcriptional analysis. Our work sets an example to apply hiPSC-based compound screening to DMD field, which overcomes the limitation of other DMD models and provide a platform for future muscular dystrophy drug discovery.

68

Analysis of The Cord Blood Hematopoietic Stem/Progenitor Cell Populations Cultured In Serum-Free Medium Under Various Conditions

Ronald L. Brown, Ph.D.[1], *Grace Almeida-Porada, M.D., Ph. D.*[2], *Christopher D. Porada Ph.D.*[2], *and Valerie Fremont, Ph.D*[1].

[1]*Quality Biological, Inc., Department of Research and Development, Gaithersburg, MD, USA, 20879; and* [2]*Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina, USA, 27157*

The use of cord blood for adult transplants has been hampered by the relatively low number of hematopoietic stem cells. Using a clinical grade serum-free medium, QBSF 60, we evaluated several combinations of early acting cytokines for their ability to support the ex vivo 2-dimensional culture of cord blood CD34+ cells while maintaining their long-term engrafting ability. The cultures were first evaluated for the phenotypic expression of the CD34 cell marker and then functionally for their short-term and long-term engrafting ability using the sheep-human xenograft model. After 14 days of culture, all the cytokine combinations supported an increase in total number of CD34+ cells from the primitive progenitor cells as well as the more mature clonogenic progenitor cells. For in vivo engrafting studies, we selected a cytokine combination for its ability to yield significant numbers of the primitive progenitors without excessive differentiation. We demonstrated that the selected culture conditions support the maintenance and/or the expansion (23-fold) of the long-term engrafting HSC over two weeks of culture. Furthermore, QBSF 60 supports the 3-dimensional culture of CD34+ cells and allows for ex vivo expansion and differentiation of CD34+ stem cells. Bioengineered liver constructs made from 3D liver-extracellular-matrix (3D-ECM) seeded with either hepatoblasts, fetal-liver-derived, or bone-marrow derived stromal cells, yielded optimal growth in QBSF 60 Medium. The 3D-ECM structures led to the maintenance of a more primitive subpopulation of hematopoietic stem and progenitor cells when cultured in QBSF 60 Medium. These results suggest that it is feasible to culture and expand cord blood CD34+ cells under clinically relevant conditions.

69

Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

Yingchao Xue[1], *Xiping Zhan*[8], *Senthilkumar S. Karuppagounder*[2,4,9], *Shuli Xia*[1,2], *Valina L. Dawson*[2,3,4,6,9], *Ted M. Dawson*[2,3,4,7,9], *John Laterra*[1,2,3,5] *and Mingyao Ying*[1,2]

[1]*Hugo W. Moser Research Institute at Kennedy Krieger, Baltimore, Maryland, USA ;* [2]*Department of Neurology;* [3]*Department of Neuroscience;* [4]*Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering* [5]*Department of Oncology;* [6]*Department of Physiology Department of Pharmacology and Molecular Sciences;* [7]*Johns Hopkins University School of Medicine, Baltimore, Maryland, USA;* [8]*Department of Physiology and Biophysics, Howard University, Washington, DC, USA;* [9]*Adrienne Helis Malvin Medical Research Foundation, New Orleans, Louisiana, USA*

Proneural transcription factors (TFs) drive highly efficient differentiation of pluripotent stem cells to lineage-specific neurons. However, current strategies mainly rely on genome-integrating viruses. Here, we used synthetic mRNAs coding two proneural TFs (Atoh1 and Ngn2) to differentiate iPS cells (iPSCs) into midbrain dopaminergic (mDA) neurons. mRNAs coding Atoh1 and Ngn2 with defined phosphosite modifications led to higher and more stable protein expression, and induced more efficient neuron conversion, as compared to mRNAs coding wild-type proteins. Using these two modified mRNAs with morphogens, we established a 5-day protocol that can rapidly generate mDA neurons with >90% purity from normal and Parkinson's disease iPSCs. After in-vitro maturation, these mRNA-induced mDA (miDA) neurons recapitulate key biochemical and electrophysiological features of primary mDA neurons and can provide high-content neuron cultures for drug discovery. Proteomic analysis of Atoh1-binding proteins identified the non-muscle Myosin II (NM-II) complex as a new binding partner of nuclear Atoh1. The NM-II complex, commonly known as an ATP-dependent molecular motor, binds more strongly to phosphosite-modified Atoh1 than the wild type. Blebbistatin, an NM-II complex antagonist, and bradykinin, an NM-II complex agonist, inhibited and promoted, respectively, the transcriptional activity of Atoh1 and the efficiency of miDA neuron generation. These findings established the first mRNA-driven strategy for efficient iPSC differentiation to mDA neurons. We further identified the NM-II complex as a positive modulator of Atoh1-driven neuron differentiation. The methodology described here will facilitate the development of mRNA-driven differentiation strategies for generating iPSC-derived progenies widely applicable to disease modeling and cell replacement therapy.

70

Cellular Metabolic Defects in Neurodevelopment Revealed by Single Cell Analysis in Patient-Specific Models of Kabuki Syndrome

Giovanni Carosso, Jonathan Augustin, Leandros Boukas, Ha Nam Nguyen, Gabrielle Cannon, Briana Winer, Johanna Robertson, Kasper Hansen, Loyal Goff & Hans Bjornsson

Mutations in the chromatin-modifying gene KMT2D are causative in 70% of Kabuki syndrome (KS) cases, in which reduced intellectual capacity beginning in early childhood is thought to arise from dampened control of the epigenome. This loss of gene expression control could manifest at any number of targets genome-wide in a variety of cell types, and has been shown to negatively impact adult neurogenesis in a mouse model, but KS-specific changes in cellular networks during neurodevelopment have yet to be discovered. We genetically reprogrammed KS patient skin cells into induced pluripotent stem cells (iPSC), enabling generation of the first human neural progenitor cell (NPC) model of KS. Compared to healthy controls, these cells show restricted proliferation and altered cell cycle progression, as well as higher rates of apoptotic cell death. Because developing NPCs exist along a heterogeneous spectrum of differentiation stages, we leveraged the powerful resolution of single cell expression profiling to identify the precise states of maturation and cell cycle occupancy of individual cells following neural induction from iPSCs. Surprisingly, we found that KS NPCs exhibit transcriptional hallmarks of precocious differentiation, with a ~20-fold increased density of cells at the most mature NPC stages, and this trend was confirmed at the protein level by flow cytometric analyses. The most transcriptionally blunted gene networks in KS NPCs govern the metabolic processes of glycolysis, protein quality control, and oxygen sensing, while protein synthesis, mRNA degradation, and apoptotic execution pathways were markedly increased. By separating NPCs into subsets along a differentiation trajectory, we specify energy-related metabolic dysregulation as a principal driver of KS gene expression in early NPCs, while mature NPC differences are biased toward mRNA degradation and ribosomal pathways. Extending these findings with CRISPR/Cas9 gene editing in an isogenic hippocampal neuron KS model, we identify concordant metabolic expression abnormalities, more precisely define a cell cycle arrest during mitotic division, and observe accumulation of misfolded protein aggregates. Identification of cell context-specific, genome-wide KMT2D binding sites refined a list of KS transcriptional targets heavily biased toward cellular oxygen sensing defects, and functional experiments demonstrate genotype-dependent effects of hypoxia on cell death induction in KS cells. Finally, we test our findings in vivo in a Kmt2d mouse model, measuring gene expression in proliferating hippocampal precursors to reveal concordant KS-associated gene expression profiles, similar mitotic division arrest, and depletion of the hippocampal adult stem cell pool. Together, our findings support a model in which altered expression of KMT2D-controlled cellular metabolic gene targets underlies an accelerated maturation of, and subsequent depletion in, pools of neural precursor cells in KS. More broadly, we demonstrate the investigative power of single cell resolution in highly heterogeneous cell contexts toward identification of disease-driving molecular mechanisms for therapeutic development in neurodevelopmental disorders.

71

Closed Systems Enabling Commercially-Viable Stem Cell Manufacturing

Jon Rowley, PhD, RoosterBio, Inc., Frederick, MD 21703, USA

The need for affordable production of cells, including human Mesenchymal Stem Cells (hMSC), in closed scalable systems has been identified as a “Current Challenge” in the Technology Roadmap established by the NIST-funded Cell Manufacturing Consortium. Additionally, hMSCs have been identified as a key critical raw material for many regenerative medicine products as reported in over 800 clinical trials with an estimated 60 Billion cells necessary for manufacture per clinical trial (Olsen, et al 2018). Therefore, a critical bottleneck impeding the growth of Regenerative Medicine is the availability of cGMP-compliant hMSC for clinical development pathways. Hence there is an immediate need to develop scalable platform systems that simplify the production of cGMP-compliant stem cells and radically reduce clinical translation timelines. The objective of this work specifically focuses on one critical component of a complete scalable closed system: the development and qualification of a functionally closed version of bone marrow derived hMSC working cell banks for use in scalable stem cell manufacturing. To achieve this goal in AIM 1, we developed the documents and production chain of closed bag systems containing a 100M cell hMSC working cell bank. This included the evaluation and validation of multiple commercially available cryobags as well as sourcing and customization of a filling manifold system for product production. An optimized controlled rate freezing protocol was evaluated for final product manufacturing, based on the post thaw viability, viable cell concentration, recovered total volume, and 5 day hMSC expansion profiles. Following product development optimization, AIM II of this study was to complete and document a comparability study between the closed system bagged hMSCs with standard open system cryovials, in 2 half-scale and 1 full-scale production runs. The comparability included assays focusing on cell viability, hMSC identity, cell recovery, as well as on characterization of hMSC differentiation potential, cytokine secretion profiles, and immunomodulatory potential. Completion of both AIM I and II will result in a final Product/Process Development Report with full comparability package to support downstream product documentation. Within 6-12 months of completing this proposal, RoosterBio expects to bring to market the novel closed system hMSC working banks, labeled ‘For Further Manufacturing’. This project supported by the MSCRF/TEDCO resulted in continued job growth within the state of Maryland as well as a direct impact for RoosterBio through in-state expenditures to support this work. The final product will shave several years and millions of dollars off the typical Regenerative Medicine product development timelines along with having a direct downstream impact through the application of the developed processes and knowledge to develop further new and innovative products. In part due to the support of MSCRF and TEDCO RoosterBio is well poised to be a leading manufacturer for Regenerative Medicine for years to come.

72

Manganese-enhanced MRI for Interrogating Astrocyte Replacement in A Mouse Model of Amyotrophic Lateral Sclerosis

Mehreen Kouser[1] , Jean-Philippe Richard[2], Michael T. McMahon[3], Nicholas J. Maragakis[2], and Jeff W.M. Bulte[1,3]

[1]Johns Hopkins University School of Medicine, The Russell H. Morgan Department of Radiology and Radiological Science, Baltimore, MD; [2]Johns Hopkins University School of Medicine, Department of Neurology and Neurosurgery, Baltimore, MD; [3]Johns Hopkins University School of Medicine, Kennedy Krieger Institute, F.M. Kirby Center for Functional Brain Imaging, Baltimore, MD

Amyotrophic Lateral Sclerosis (ALS) is a devastating disease with near 100% mortality. An important role has been assigned to (over)activated astrocytes. Given the few available treatment options, stem cell therapy is now actively being pursued as a new treatment paradigm, aimed at either immunomodulation (using mesenchymal stem cells - MSCs), astrocyte replacement (using glial progenitors), or motor neuron restoration, with several clinical trials currently in progress. Our goal is to better understand the mechanisms underlying astrocyte activation that creates a toxic environment for motor neurons causing their death. We aim to replace host glial-restricted progenitors (GRPs) to preserve the neuronal environment using intraspinal transplantation and to develop novel imaging biomarkers that can report not only on the fate of transplanted cells but also on changes in the ALS host environment. To this end, we investigated the activation state of host cells in vivo using manganese ($MnCl_2$) -enhanced magnetic resonance imaging MRI (MEMRI). We used transgenic SOD1G93A male mice from JAX as the model for ALS and compared them to the age and sex matched wild type controls. Both SOD1G93A and WT mice were randomly assigned to either the saline or $MnCl_2$ group resulting in four groups total which were, WT-Saline, WT-$MnCl_2$, SODG93A-Saline, and SODG93A-$MnCl_2$. All mice were imaged at pre-symptomatic (60 day), onset (90 day), and post-symptomatic (120 day) stages of ALS. Images of the lumbar region of the spinal cord were obtained before and 24 hours after i.p. injection of a fresh solution of 30 mM $MnCl_2$ at 0.4 mmol/kg body weight in saline at the above stated ages. Our data show that both WT and SOD1G93A mice exhibit an increase in T1w image contrast 24 h after $MnCl_2$ injection. Moreover, in contrast to our hypothesis, there is no observable T1w image contrast difference as the disease progresses in grey or white matter of the spinal cord. Immunohistological analysis of neuroinflammation, motor neuron degeneration, and astrocyte pathology is currently underway. Meanwhile we are also exploring other brain regions where we may be able to observe activated host astrocytes using MEMRI.