MSCRF

Maryland

STEM CELL RESEARCH FUND

Annual Report

2017

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Congratulations to the 2017 & 2018 MSCRF Grant Award Recipients

FY 2017 MSCRF Awards:

Clinical Grant Awards:

Joshua M. Hare, M.D.
Longeveron, LLC.
Mesenchymal Stem Cell Therapy and Surgical Palliation for Hypoplastic Left Heart Syndrome (HLHS)

Gurdyal Kalsi, M.D.
TissueGene, Inc.
Assessment of the Efficacy of Cell Therapy in Treating Synovitis Using Contrast Enhanced MRI in a Clinical Study of Knee Osteoarthritis

Commercialization Grant Awards:

Ha Nam Nguyen, Ph.D.
3Dnamics Inc.
Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening and Toxicity Testing

Madhusudan Peshwa, Ph.D.
MaxCyte, Inc.
Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)

William Rust, Ph.D.
Seraxis, Inc.
Long-term Function of Stem Cell Grafts for Insulin-Dependent Diabetes

Chengkang Zhang, Ph.D.
Propagenix, Inc.
Building Commercial Path for EpiX™ Technology - A Breakthrough in Expanding and Utilizing Tissue-Resident Stem Cells

Validation Grant Awards:

Sharon Gerecht, Ph.D.
Johns Hopkins University
Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Sunjay Kaushal, Ph.D.
University of Maryland, Baltimore
Neonatal Cardiac Stem Cells for Heart Regeneration

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Lineage Reprogramming of human Fibroblasts into Oligodendrocyte Progenitor Cells

Discovery Grant Awards:

Jeff Bulte, M.S., Ph.D.
Johns Hopkins University
Non-Invasive Imaging of Hydrogel Scaffold Biodegradation & Cell Survival

Linzhao Cheng, Ph.D.
Johns Hopkins University
Human iPSC-Derived Extracellular Vesicles: Unique Properties & Potential for Tissue Regeneration

Ivy Dick, Ph.D.
University of Maryland - Baltimore
Developing a Novel Treatment Strategy for Timothy Syndrome

Aaron James, M.D., Ph.D.
Johns Hopkins University
Regional Specification of Bone-Associated Perivascular MSC

David Kass, M.D.
Johns Hopkins University
Conditional Power Switch for Stem Cell-Derived Cardiomyocytes

Gabsang Lee, Ph.D.
Johns Hopkins University
Pharmacological Cues to Expand Functional Human PAX7::GFP+ Skeletal Muscle Stem/Progenitor Cells

Discovery Grant Awards *(Cont'd)*:

Brady Maher, Ph.D.
Lieber Institute for Brain Development
Use Human Cellular Models of Pitt-Hopkins Syndrome to Study Neuronal Development and Validate Therapeutic Targets

Michael Nestor, Ph.D.
The Hussman Institute for Autism
Establishing a 3D Based High-Content Screening Platform for Cellular / Phenotypes in Autism

Linda Resar, M.D.
Johns Hopkins University
Developing Stem Cell Technology for Tissue Repair and Goblet Cells for Ulcerative Colitis Patients

Piotr Walczak, M.D. Ph.D.
Johns Hopkins University
Inducing Immunotolerance of Myelinating Progenitor Cells Transplanted into the Brain of Immunocompetent Mice

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Highly Efficient Conversion of iPS Cells to Motor Neurons and Oligodendrocytes by Synthetic Modified mRNAs

Post-Doctoral Fellowship Grant Awards:

Qin Bian, Ph.D.
Johns Hopkins University
Direct Specification of Articular Chondrocytes from iPSC-Derived Lateral Plate Mesoderm

Dongwon Kim, Ph.D.
Johns Hopkins University
Postdoctoral Training Towards Independence: Testing of Skin Stem Cells to Modify Skin Identity

Josephine Lembong, Ph.D.
University of Maryland - College Park
Stem Cell Expansion and Differentiation in Bioreactors via Coupling of Substrate Curvature and Shear Stress

Joseph Mertz, Ph.D.
Johns Hopkins University
Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-derived Retinal Ganglion Cell

Fahimeh Mirakhori, Ph.D.
Johns Hopkins University
Establishment of All Human Schwann Cell In-Vitro Myelination

Nikhil Panicker, Ph.D.
Johns Hopkins University
Activation of the NLRP3 Inflammasome in Human Dopamine Neurons as a Consequence of Parkin Dysfunction

Marco Santoro, Ph.D.
University of Maryland - College Park
Development of Tissue-Engineered Vascularized Scaffolds via 3D Printing of Endothelial/Stem Cells

Congshan Sun, Ph.D.
Johns Hopkins University
hiPSC Based Compound Screening for Treatment of Duchenne Muscular Dystrophy (DMD)

Aline Thomas, Ph.D.
Johns Hopkins University
Development of Stem Cell Therapies for Multiple Sclerosis using Non-Invasive Biomarkers

Zhao Wei, Ph.D.
Johns Hopkins University
3D Printing Vascularized Cardiac Constructs

FY 2018 MSCRF Awards: *(First Funding Cycle)*

Commercialization Grant Awards:

Hai-Quan Mao, Ph.D.
LifeSprout, LLC
Delivery of MSCs to Enhance the Replacement and Regeneration of Soft Tissue

Jonathan Rowley, Ph.D.
RoosterBio, Inc.
Closed Systems Enabling Commercially-Viable Stem Cell Manufacturing

Validation Grant Awards:

Chulan Kwon, M.S., Ph.D.
Johns Hopkins University
Developing Adult Cells from iPSCs

Elias Zambidis, M.D., Ph.D.
Johns Hopkins University
MoroPLUR: A Defined Feeder-Free Medium for Enhancing Functionality of Human Pluripotent Stem Cells

Completed: FY 2014 MSCRF Award:

A-Lien Lu-Chang, Ph.D.
University of Maryland, Baltimore
Exploratory Award
The Effects of Histone Deacetylation and DNA Demethylation on Somatic Cell Reprogramming

Jonathan Dinman, Ph.D.
University of Maryland, College Park
Exploratory Award
Directed delivery of Therapeutic RNAs into Hematopoietic Stem-Progenitor Cells

Miroslaw Janowski, M.A., M.D., Ph.D.
Johns Hopkins University
Exploratory Award
Magnetic Resonance Imaging of Myelination by Transplanted Glial Restricted Precursor Cells

Manoj Kumar, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Ted Dawson, M.D., Ph.D.
Human Dopaminergic Neuronal Loss Due To Parkin Insufficiency: Relevance to Parkinson's Disease

Nicholas Maragakis, M.D.
Johns Hopkins University
Exploratory Award
IPSC-Derived Neurons from Amyotrophic Lateral Sclerosis Patients to Study Disease Progression

Ke Ren, Ph.D., M.D.
University of Maryland, Baltimore
Investigator Initiated Award
Mesenchymal Stem Cells for Chronic Pain Therapy

Lipeng Tian, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Yoon-Young Jang, M.D., Ph.D.
Human Stem Cell based Model of Alcoholic Liver Disease for Regenerative Therapy

Stephen Wolpe, Ph.D.
Orgenesis Maryland, Inc.
Pre-Clinical Award
Autologous Insulin Producing (AIP) Cells for Diabetes

Zijun Zhang, M.D., Ph.D.
MedStar Health Research Institute
Exploratory Award
Enhancing the Incorporation of Bone Allograft with Circulating Mesenchymal Stem Cells

Completed: FY 2015 MSCRF Awards:

Srinivasa Dalta, Ph.D.
University of Maryland, Baltimore
Post-Doctoral Fellowship Award
Mentor: Sunjay Kaushal, M.D.
Allogeneic Safety Testing of c-Kit+ Cardiac Stem Cells

Ted Dawson, M.D., Ph.D.
Johns Hopkins University
Exploratory Award
Development of a Chimeric Human Mouse Model of Parkinson's Disease

Peter Johnston, Ph.D.
Johns Hopkins University
Exploratory Award
Cell Impregnated Nanofiber Stent Sleeve for Peripheral Vascular Repair

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Exploratory Award
Regulation of Oligodendrocyte Identity by MicroRNA Networks

Ileana Lorenzini, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Rita Sattler, Ph.D.
Role of Structural and Functional Changes of Dendritic Spines in Patient-Derived C9ORF72 iPS Neurons

Hideki Uosaki, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Chulan Kwon, M.S., Ph.D.
MicroRNA-based Maturation of Cardiomyocytes Derived from Human Pluripotent Stem Cells

Jiou Wang, M.D., Ph.D.
Johns Hopkins University
Exploratory Award
Pathogenic Mechanisms and Intervention Targets in Neuro-Degenerative Disease ALS/FTD

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Exploratory Award
Highly Efficient Conversion of Human iPS Cells to Dopaminergic Neurons by Synthetic Modified mRNAs

Sooyeon Yoo, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Seth Blackshaw, Ph.D.
Adult Hypothalamic Neurogenesis and Regulation of Feeding and Metabolism

Ki-Jun Yun, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Hongjun Song, Ph.D.
Modeling Neurodevelopmental Defects in Psychiatric Disorders using iPSC-Derived 3D Cerebral Organoid

Completed: FY 2016 MSCRF Awards

Allison Bond, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Guo-Li Ming, M.D., Ph.D.
Evaluating the Impact of Genetic Risk Factors for Psychiatric Disorders on Interneurons

Dhruv Vig, Ph.D.
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentors: Sharon Gerecht, Ph.D. & Sean Sun, Ph.D.
Geometric Cues in the Establishment and Maintenance of Heterogeneous Stem Cell Colonies

Kathryn Wagner, M.D., Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Exploratory Award
A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation (Continuation)

Maryland Stem Cell Research Commission

David Mosser, Ph.D. – Chair
(Appointed by the University System of Maryland)
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park.

Debra Mathews, Ph.D., MA - Vice Chair
(Appointed by Johns Hopkins University)
Assistant Director for Science Programs, Johns Hopkins Berman Institute of Bioethics; Assistant Professor, Dept. of Pediatrics, Johns Hopkins School of Medicine.

Rachel Brewster, Ph.D.
(Appointed by the University System of Maryland)
Associate Professor; Biological Sciences University of Maryland, Baltimore County

Margaret Conn Himelfarb
(Appointed by the Governor)
Health Advisory Board and Institutional Review Board, Johns Hopkins Bloomberg School of Public Health; Embryonic Stem Cell Research Oversight Committee, Johns Hopkins School of Medicine.

Diane Hoffmann, M.S., J.D.
(Appointed by the University System of Maryland)
Professor of Law, Director Law & Health Care Program, University of Maryland School of Law

Haig H. Kazazian, Jr., Ph.D.
(Appointed by Johns Hopkins University)
Professor of Pediatrics McKusick-Nathans Institute of Genetic Medicine

Marye D. Kellermann, RN, Ph.D.
(Appointed by the Speaker of the House of Delegates)
Patient Advocate; President, Educational Entities; Enterprises NECESSARY NP Reviews & NECESSARY Workshops.

Sharon Krag, Ph.D.
(Appointed by Johns Hopkins University)
Professor Emerita Department of Biochemistry & Molecular Biology, Johns Hopkins University Bloomberg School of Public Health.

Linda Powers, J.D.
(Appointed by the President of the Senate)
Managing Director of Toucan Capital, Early & Active Supporter of Biotech Companies

Rabbi Avram I. Reisner, Ph.D.
(Appointed by the Governor)
Rabbi of Congregation Chevrei Tzedek, Baltimore, Maryland.

Ira Schwartz, Esq.
Senior Assistant Attorney General & Counsel to the Maryland Technology Development Corporation (TEDCO)

Curt Van Tassell, Ph.D.
(Appointed by the Speaker of the House of Delegates)
Research Geneticist, USDA-ARS, Beltsville, MD

Bowen P. Weisheit, Jr.
(Appointed by the Governor)
Patient Advocate; Board member of the Maryland Chapter of Cystic Fibrosis Foundation; & Attorney, Law Office of Bowen Weisheit, Jr.

10 Years of Research Innovation and Cures

Over the last ten years we, at the Maryland Stem Cell Research Fund (MSCRF), have been at the forefront of innovation- funding the most ground-breaking research and the most innovative companies in our state as well as our nation. Last year we launched a new initiative we called Accelerating Cures. With this new initiative we aimed to innovate and discover but also help these new ideas mature and transition from the bench into commercial products and cures in the marketplace. Although we are now only 18 months into this enterprise and only 6 months into our first funded projects, we are already seeing the initial results.

In a short time, we have identified, helped and funded over 13 new technologies from our Universities that are looking for market validation. We have created a community of over 50 companies in the cell therapy, stem cell or regenerative medicine field. In addition, we have had in depth discussions with 28 companies about potential opportunities and have funded 8 new companies.This overwhelming interest and excitement from our community endorses our new strategy and our commitment to continue along this path of creating a sustainable market and delivering cures.

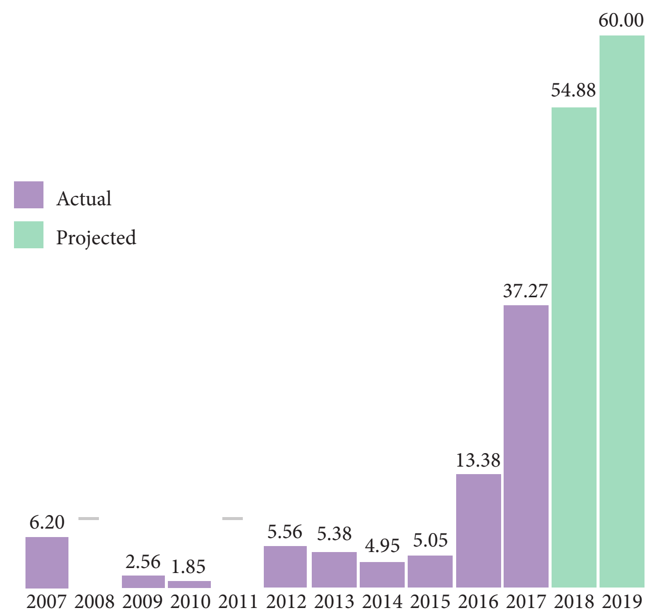
Economic Contributions of MSCRF

A recent report on the Economic Contributions of the Maryland Stem Cell Research Fund by the Sage Policy Group found that Business sales in Maryland have been bolstered by more than $286 million as a result of the MSCRF, or by more than two times State contributions.

2017 Dollars	Jobs (FTEs)	Employee Compensation	Business Sales
Direct Effects	495	$57,091,212	$146,180,487
Indirect Effects	446	$28,818,832	$72,341,102
Induced Effects	464	$22,789,427	$67,931,084
Total	1,405	$108,699,471	$286,452,673

Growth of Stem Cell Research Commercialization

The report also found that in 2017 alone, private companies accounted for $2.7 million in awards or 31.9 percent of total MSCRF funding.The overall budget projected towards commercialization would go to as high as 60% in the upcoming years.



For full access for the economic study and more information about our funded projects and companies please check our website www.mscrf.org

Stem Cell Symposium and Partnering

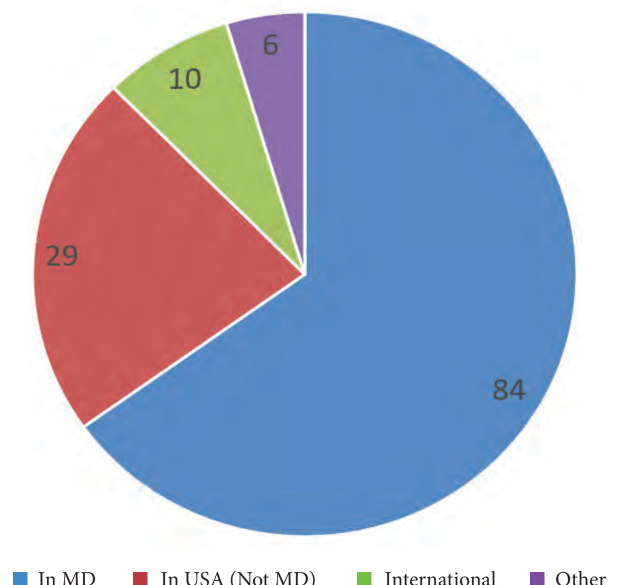
This year the annual stem cell symposium was held along with the TEDCO Entrepreneur Expo. This combined event attracted over 800 attendees and allowed stem cell scientists from across Maryland to interact with companies, investors and entrepreneurs in the region to stimulate translation of research ideas into commercial products and cures.

The day-long Stem Cell Track featured 15 speakers from local universities, university start-ups as well as established companies in Maryland. The program, like our Accelerating Cures initiative, covered a spectrum of cutting-edge topics spanning discovery, commercialization and clinical trials. Our eminent speakers presented progress in Stem cells and disease modeling, Tissue Engineering and clinical applications, Advances in stem cell product development and Stem cells road to clinic. 86 scientists presented posters on their research and development. Overall, these interactions inspired innovation and collaborations which will propel this field forward in our region.

Training Future Scientists

In addition to supporting faculty and companies MSCRF has, over the past several years, provided fellowship training grants for post-doctoral trainees at our Universities. Over the last year, as part of a new initiative, we also started a new facilitated round table discussion group for our funded post-docs. The goal of this round table is to build companionship and help our awardees establish a strong foundation in translational regenerative medicine and increase future commercialization of their ideas and innovations. Over the last 9 years of the post-doctoral program we were able to attract and retain the best and the brightest of our nation's researchers here to Maryland. The success of the program is beyond expectations, with over 65% of them continuing to work in our state universities and industries.

2008 - 2017 Postdocs



Accelerating Cures

Another important aspect of the Accelerating Cures initiative is the urgency we introduced to the community. Our review cycle is less than two months; all of our awards are now for a period of 2 years or less, and we added a second cycle within the same fiscal year to allow our Validation and Commercialization applicants a faster path to market.

ACCELERATING CURES

Discovery:

Grants for new innovative ideas Research- requests up to $345,000 for up to two years.

Validation:

Grants to foster the transition of promising stem cell technologies having significant commercial potential from Universities and research labs, to the commercial sector - requests up to $230,000 for up to 18 months

Commercialization:

Grants for the creation of Start-up companies or new technologies developed in Maryland based companies - requests up to $300,000 for up to 12 months.

Clinical:

For conducting clinical trials in Maryland using human stem cells to advance medical therapies -request up to $750,000 for up to two years and requires a 1:1 match of non-state money.

Post-Doctoral Fellowship:

Grant to support exceptional post-doctoral fellows who wish to conduct research in academia or in industry in the State of Maryland. Each Fellowship will be up to $65,000 per year, for up to two years.

Clinical Research Grant Awards

Joshua Hare, M.D.
Longeveron, LLC
Award Amount: $750,000
Disease Target: Hypoplastic Left Heart Syndrome (HLHS)

Mesenchymal Stem Cell Therapy and Surgical Palliation for Hypoplastic Left Heart Syndrome

How clinical practice and treatment of immunosenescence in Aging Frailty will be advanced by the proposed research. If successful, this proposed clinical therapy would advance clinical treatment for HLHS by setting a new standard of care using combinatorial intervention of LMSCs and standard surgical techniques. We anticipate that treated HLHS patients will have significantly improved long-term outcomes, including decreased mortality rate, decreased need for cardiac transplantation, improved circulation, and improved quality of life. Since LMSCs are an allogeneic therapy that could be obtained "off-the-shelf" once FDA approved, this treatment option would become generally affordable and available. How the proposed research may contribute to new medical treatments or interventions. The proposed study would be the first such study using allogeneic MSCs to treat infants. If successful, this pioneering research could pave the way to broader applications of LMSC therapy to congenital disorders. This could potentially revolutionize medicine by combining cellular therapy with surgical interventions to dramatically improve patient survival and recovery. How the proposed research will translate prior research results into new medical therapies or test new therapies in human patient. We have significant prior data demonstrating the clinical efficacy and safety of MSC therapy for adult cardiac patients. The proposed research herein is a natural extension of these results, applied for the first time ever to infant cardiac patients. Projected plan and time line. We anticipate completing this study in 3 years. During the first year, we will perform the Safety Run-In phase of this study. During years 2 and 3, we will perform the randomized. A timeline and milestones are provided in the Gantt chart in the Research Plan.

Gurdyal Kalsi, M.D.
TissueGene, Inc.
Award Amount: $750,000
Disease Target: Osteoarthritis (OA)

Assessment of the Efficacy of Cell Therapy in Treating Synovitis Using Contrast Enhanced MRI in a Clinical Study of Knee Osteoarthritis

TissueGene, Inc. is a Maryland based clinical-stage biopharmaceutical company focused on developing novel regenerative therapies in the field of cell therapy. TG-C is the lead therapeutic product that TissueGene is developing for the treatment of osteoarthritis (OA), a highly significant problem in the fast growing aging society. Currently, it is estimated that osteoarthritis affects over 27 million adults in the US and over 151 million adults worldwide. Available treatments of knee OA help reducing adverse symptoms, but not curing the disease. Patients with advanced OA may require invasive treatment such as knee arthroplasty. TG-C's design is based on an allogeneic (donor) cell therapy involving human chondrocytes (cartilage cells) that are genetically manipulated to express the therapeutic growth factor TGF-β1. TG-C exerts its therapeutic effect in osteoarthritis through the regeneration of knee cartilage in reduction in inflammation. The TG-C treatment is minimally invasive (a single intraarticular injection) and may delay or prevent arthroplasty. In Phase 1 and 2 clinical trials, TG-C treatment exhibited a good safety profile and sustained positive effects on functional outcomes and cartilage growth. The proposed Clinical Phase 3 trial is a study focusing on confirming prior findings on the symptomatic improvements and providing additional evidence of structural effects. This is an advanced study over the past trials in terms of the employment of high resolution imaging techniques and will also help us understand effects of TG-C treatment on other aspects of OA such as synovitis through additional imaging modalities such as contrast-enhanced MRI. Osteoarthritis is a chronic disease caused by the progressive degeneration of articular cartilage and is characterized by joint pain, stiffness, and limited movement. The TG-C treatment has several advantages over current therapies, including 1) the TG-C treatment is minimally invasive, requiring only a single intraarticular injection, 2) TG-C is a gene-modified cellular therapy that exhibits regenerative capabilities due to the cells and exposure to the expressed therapeutic protein at a specific therapeutic level and duration of time, and 3) the cells in TG-C are selected and screened for a variety of cellular expression characteristics to enhance efficacy and minimize the patient's immune response to the injected cells. Over a 2-year period, we will focus on: 1) human subjects with primary osteoarthritis of the knee will be recruited and treated with TG-C; 2) they will be evaluated for effects on pain, function and joint structure using standard methodology; and within this study, a subgroup of 30 subjects (10 by placebo treatment) will be evaluated with high resolution contrast-enhanced MRI to assess the effects on synovitis, one aspect of OA. The MRI images will be examined by an experienced radiologist (blinded to treatment) to evaluate whether there are statistically significant impacts observable from the TG-C treatment on joint structure, cartilage thickness, bone marrow lesions, and in particular, synovitis. Ultimately, this study will further demonstrate the safety and effectiveness of TG-C.

Discovery Research Grant Awards

Jeff Bulte, M.S., Ph.D.
Johns Hopkins University (JHU)
Award Amount: $345,000
Disease Target: Amyotropic Lateral Sclerosis (ALS)

Non-Invasive Imaging of Hydrogel Scaffold Biodegradation and Cell Survival

Hydrogel scaffolds are increasingly being used as tissue-mimicking materials and as vehicles to improve transplanted stem cell retention and survival. We have recently developed a new chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI) method that is able to probe the in vivo stability and gelatin decomposition of implanted composite hyaluronic acid (HA) hydrogels in a “label-free” fashion. Compared to naked cells, we found that transplanted neural stem cells showed improved survival when hydrogel scaffolding was applied. A major question that remains is the optimal mechanical properties of the hydrogel, and how this relates to cell survival. At the one hand, for initial structural support, the gels should not decompose too fast, but at the other hand they should at some point decompose to allow transplanted cells to grow out and integrate with the surrounding host tissue. Our aim is to synthesize a range of composite dual-labeled near-infrared (NIR)-HA hydrogels with different compositions and stabilities, to image their stability properties in vivo, and to correlate the CEST/NIR optical imaging findings with cell survival as assessed using bioluminescent imaging (BLI) as conventional readout. Using herpes simplex virus 1 thymidine kinase (HSV1-tk) as a new CEST MRI reporter gene, we will also investigate whether or not CEST MRI is able to probe in vivo cell survival simultaneously. We have chosen to apply this approach to transplantation of glial-restricted precursor cells (GRPs) in a transgenic amyotropic lateral sclerosis (ALS) mouse model, as we have found that transplanted naked cells without hydrogel scaffolding survive poorly in the hostile ALS tissue environment.

Linzhao Cheng, Ph.D.
Johns Hopkins University (JHU)
Award Amount: $345,000
Disease Target: Bone Marrow Transplantation (BMT)

Human iPSC-Derived Extracellular Vesicles: Unique Properties & Potential for Tissue Regeneration

In this innovative discovery project, we will explore if extracellular vesicles (EVs) released by human stem cells will be able to substitute or enhance infused medicinal cells in autologous and allogenic cell therapies for tissue regeneration. An increasing body of evidence suggests that the observed medicinal effects after transplanting or transfusing mesenchymal stem cells (MSCs) are mainly due to the paracrine stimulation by MSCs in vivo. It is now widely accepted that MSCs produce bioactive molecules acting on host cells, instead of significant integration into the targeted issue by themselves or their differentiated progeny cells. Moreover, recent studies indicated that EVs secreted by MSCs are most likely responsible for MSC’s transient paracrine effects observed in several animal models and in clinical trials. In this project, we will focus on examining if EVs made by MSCs established from human adult marrow (BM MSCs) and induced pluripotent stem cells (iPSC- MSCs) will be able to aid marrow and hematopoietic cell recovery after irradiation damage. In addition, we will also use EVs secreted from human iPSCs, which have greater self-renewal and developmental potential, and can be expanded infinitely under a completely defined culture medium. EVs can be produced from human iPSCs in greater numbers with cargos containing a different combination of proteins and RNAs than EVs from MSCs. Our preliminary data suggest that EVs from human iPSCs are very stable. We hypothesize that EVs that are made by stem cells with different protein and RNA contents will have also different biological activities as compared to EVs made by non-stem cells. After essential characterization of their physical and biological properties, we will first investigate if EVs from human MSCs and iPSCs may aid marrow and hematopoietic cell recovery after irradiation damage. If so, infusion of EVs may improve the outcome of bone marrow transplantation (BMT) that is increasingly used for treating non-malignant and malignant diseases. This discovery project will determine if EVs form human iPSCs as well as MSCs provide a novel “cell-free” modality for aiding tissue regeneration after BMT, irradiation damage or other types of tissue injuries.

Ivy Dick, Ph.D.
University of Maryland, Baltimore
Award Amount: $345,000
Disease Target: Timothy Syndrome

Developing a Novel Treatment Strategy for Timothy Syndrome

Back and leg pain due to intervertebral disc (IVD) degeneration are common symptoms associated with aging. While surgical treatment and an appropriate rehabilitation plan may offer relief for acute symptoms, degenerative changes often prevent recovery to the premorbid condition and usually lead to lifelong morbidity and premature retirement, and as a result new treatment strategies aimed at the full restoration of the IVD are highly desired. Stem cell therapy is a very promising strategy to address these types of disabilities, and Human Mesenchymal stem cells (hMSCs) have a demonstrated potential to differentiate toward multiple types of connective tissue cells (including bone and cartilage) and represent an excellent option for treatment of degenerative disc disease. The use of tissue composites to support hMSCs has drawn quite a bit of attention. Information about long-term survival of transplanted hMSCs is also critical for outcome evaluation, and in a clinical setting, this can only be achieved using non-invasive imaging such as MRI. Capitalizing on our decade-long experience as leaders in the field of MRI contrast agent development and our previous results which demonstrated the feasibility of incorporating MRI pH sensors into composites to report on cell death, we have developed a method for monitoring the delivery of labeled cell composites in real-time using MRI, enabling the interactive adjustment of cell delivery and monitoring of their survival. In Specific Aim 1 we will optimize two formulations of cell composites, the first based on imidazoles and a second based on salicylates synthesized in our lab. We will then get a preliminary evaluation of their utility for monitoring hMSC survival in mice. The results of our novel MRI-based sensors will be validated using firefly luciferase expression to determine hMSC survival. While there is tremendous progress in stem cell biology, currently used methods of cell delivery lack precision and accuracy allowing only proof-of-concept preclinical studies. Truly translational approaches need to emphasize the process of stem cell delivery so the procedure is performed in a reliable and reproducible fashion, yielding consistent results. Indeed, high variability has been observed in clinical trials employing stem cells, which translates to a reduction in statistical significance. The complexity of cell transplantation procedures dramatically increases when translating from rodent studies through large animal studies to human trials. To test our new MRI detectable stem cell supporting material further, an appropriate animal model is required, and we have selected a pig model of IVD. Once the best formulation has been identified, we will administer this composite into the IV space in pigs in Specific Aim 2. Half of the recipients (n=12) will not be immunosuppressed and are expected to reject the graft, while the other half (n=12) will be immunosuppressed and is expected support graft survival over a prolonged period. The difference in CEST signal for transplanted tissue composites between non-immunosuppressed and immunosuppressed pigs will be compared to evaluate this technology and post mortem analysis will be performed to confirm the fate and distribution of the transplanted hMSCs.

Aaron James, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Bone / Cartilage Disorders

Regional Specification of Bone-Associated Perivascular MSC

The vasculature connects all human tissues. Beyond being a conduit for gas exchange, the vasculature houses mesenchymal progenitor cells that actively participate in tissue repair. The human pericyte has been recognized as bona fide mesenchymal stem cell (MSC) via expression of MSC markers, ability for self-renewal, and multilineage mesodermal differentiation potential. Despite the ubiquitous distribution of pericytes, their tissue specific functions remain shrouded in mystery. Data to date support the notion that pericytes have organ-specific repair functions while maintaining MSC properties. For example, pericytes around articular cartilage are primed for chondrogenesis, while dental pulp pericytes spontaneously regenerate dentin. Understanding how pericytes regenerate their micro-environment with such remarkable fidelity is of central importance for cell-based efforts in regenerative medicine. Our research group has defined the robust regenerative potential of pericytes, focusing on the natural proclivity of pericytes to form bone. These findings have been documented across multiple small animal orthopaedic models of repair. Despite our successful efforts in pericyte-mediated bone regeneration, very little is known regarding the regional specification of bone-associated pericytes. Here, we will seek to overturn conventional thinking by examining regional differences among periosteum (PO) and bone marrow (BM) derived human pericytes. Tissue specific pericyte diversity has garnered increasing attention, but this concept is essentially unexplored in bone. Our hope is that elucidating regional specification of bone pericytes will have far reaching implications for basic knowledge in bone pathophysiology, as well as future efforts in tissue engineering. Our overall hypothesis is that pericytes from each bone-associated site will recapitulate their normal response to bone injury. Like periosteal response to injury, PO-derived pericytes will be primed for osteochondral differentiation. Like bone marrow response to injury, BM pericyte subsets will display dimorphic tendencies for fibrosis or osteogenesis. Aim 1: Define the regional specification of periosteum- and bone marrow-derived human pericytes. Aim 2: Determine how periosteum- or bone marrow-origin impacts the regenerative phenotype of human pericytes. Next, the spontaneous differentiation potential of PO- and BM-derived pericytes will be defined, using xenotransplantation assays in SCID mice. PO- and BM-derived pericytes will be derived and implanted subcutaneously. Histologic and immunohistochemical analysis will be performed to examine the resulting tissue produced by implanted pericytes. Pericytes represent a MSC population with great promise in skeletal repair. MSC/progenitor cell therapies have high potential for the treatment of common conditions such as osteoarthritis, bone fracture, non-healing skeletal defects, and fibrous non-unions. Nevertheless, the regional specification of bone pericytes and the manner in which this affects human health is unknown. The current MSCRF proposal will explore this entirely new direction, with the hopes of opening up new avenues to promote health and speed repair of the human skeleton.

David Kass, M.D.
Johns Hopkins University
Award Amount: $313,013
Disease Target: Heart Myocardial Infarction

Conditional Power Switch for Stem Cell-Derived Cardiomyocytes

Cardiac regeneration therapy is a potentially transformative approach to repair the damaged heart. Over the past 15 years, there have been many preclinical studies and dozens of clinical trials testing various stem cell-based therapies, most focusing on myocardial infarction. Their results vary considerably, and fueled suspicion that paracrine secreted factors are more likely relevant than muscle cell generation. However, this remains far from settled. A central question that remarkably has yet to be answered is if engrafted stem cells and/or progenitor cells provide meaningful force of contraction in vivo to explain improved systolic function. We have no current assay to determine this. While we can detect stem cells after transplantation or transdifferentiation in the muscle tissue, we cannot determine what they are actually doing. Our project is to equip them with a genetically encoded power switch that can be turned on and off reversibly. To generate this, we will conditionally express a small GTPase protein called Rem1, that potently inhibits the voltage-gated calcium channel Cav1.2 (ICa,L) required for contraction. A mCherry color-coded Rem1 under control of a doxycycline-inducible promoter is introduced into human ESCs at high efficiency, and we will assess the electromechanical properties of these cells grown in syncytium with non-modified cells (e.g. to confirm cell autonomy of the power switch), determine the impact of altering calcium cycling in stem cells on exosome secretion, and establish methods to differentially control these cells in vivo. We will then test their contribution to cardiac contraction in vivo using a well-established rat infarction model. This work has high impact for the field. Success will provide investigators with a new metric for contractile impact of stem cell therapy. The approach can be further developed for 3-D tissue testing, and in a mouse where inducible Rem-1 is unmasked only in native transformed or newly developed nascent myocytes. As we develop better approaches to sustaining engraftment of implanted cells, our power-switch metric could well provide a gold standard for assaying their direct contribution to heart contraction. Nothing like this currently exists, yet our work addresses a major limitation of existing stem cell research. We have some limited early data, but this is an ideal project for the Discovery Program. The investigative team is at Johns Hopkins, with valuable consultancy from Charles Murry, a leading stem cell researcher at the University of Seattle, Washington. The PI is an Outstanding Investigator (R35 recipient) of the National Heart Lung and Blood Institute, and international leader in myocardial biology, heart failure, and molecular physiology. His Co-Investigator – Chulan Kwon – is a leader in stem cell biology and together they form a unique team to accomplish the work.

Gabsang Lee, Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Duchenne Muscular Dystrophy (DMD)

Pharmacological Cues to Expand Functional Human PAX7::GFP+ Skeletal Muscle Stem / Progenitor Cells

Muscle wasting, caused by aging, genetic mutations, cancer associated cachexia, or traumatic injury, can result in significant functional impairment, and it is a challenging clinical problem placing a significant socioeconomic burden on our healthcare system. The intractability of genetic disorders in skeletal muscle and other muscle wasting conditions could be overcome with innovative approaches to derive human autologous skeletal muscle stem cells and an in depth understanding of the cellular behaviors of muscle stem cells to functionally integrate them to host populations. Our group has recently developed a novel approach to direct human induced pluripotent stem cells (hiPSCs) into functional skeletal muscle cells in a highly defined, fast, and efficient manner (without ectopic expression of myogenic transcription factors). Our approach is readily applied to Duchenne muscular dystrophy (DMD)-specific hiPSCs, and it yields patient-specific skeletal muscle cells showing DMD-relevant phenotypes, which could be reversed by gene-correction of DMD-hiPSCs. However, it is still unknown how to efficiently expand the genetically-corrected human skeletal muscle stem/progenitor cells, which is a critical step for therapeutic intervention. Using our previous chemical compound screening expertise and our skeletal muscle studies with hiPSCs, we will use PAX7 as a surrogate marker to identify small molecule(s) for expanding human skeletal muscle stem/progenitor cells of healthy and genetically-corrected hiPSCs of DMD. Our approach to robustly expand human PAX7::GFP+ cells, together with recent gene therapy efforts by other investigators, will have a profound impact on functional restoration of damaged tissues. Therefore, these studies may establish a new strategy and regimen to treat muscle wasting conditions by providing functionally competent myogenic stem cells. In addition, our approach will not only shed light on the mechanistic understanding of human muscle stemness, but it may also lead us to find drug(s) to stimulate and/or repair muscle tissue in vivo. These approaches are highly translational, which is suitable to the new vision of MSCRF/TEDCO.

Brady Maher, Ph.D.
Lieber Institute for Brain Development
Award Amount: $345,000
Disease Target: Pitt-Hopkins Syndrome (PTHS)

Use Human Cellular Models of Pitt-Hopkins Syndrome to Study Neuronal Development and Validate Therapeutic Targets

Autism spectrum disorder (ASD) is a debilitating and costly psychiatric disorder for which we have a limited understanding of its etiology and underlying pathophysiology. Current treatments involve behavioral therapies that have a limited effect and/or pharmacotherapies that are largely ineffective at treating the core deficits of the disorder. The complexity of these psychiatric disorders necessitates the need for further research into the neurobiological basis so therapeutic targets with broad applications can be discovered. There exists a strong genetic basis for ASDs through genetic linkage, association studies, and the presence of highly penetrant copy number variations that increase risk. In addition, several monogenetic mutations are associated with syndromic forms of ASD and this direct genetic association provides an entry point into developing models that offer potential insight into the underlying neurobiology. My research group has begun to make significant progress studying a syndromic form of ASD called Pitt-Hopkins Syndrome (PTHS), a relatively unstudied disorder that is characterized by developmental delays, intellectual disability, epilepsy, autistic behavior, breathing abnormalities, and gastrointestinal issues. PTHS is caused by an autosomal dominant deletion or mutation in the gene transcription factor 4 (TCF4), which plays a critical role in the development and maturation of cortical neurons. Peak expression of TCF4 transcripts and protein levels are observed during fetal cortical development and then expression subsides to lower levels during adolescence and adulthood. We have modeled PTHS using two independent rodent models and have observed that suppressing TCF4 function results in significant deficits in the intrinsic excitability and spontaneous synaptic transmission of prefrontal neurons. Molecular profiling experiments conducted in both model systems have identified a voltage-gated sodium ion channel (SCN10a) that was significantly upregulated when TCF4 was disrupted. The role for SCN10a in this excitability phenotype was validated through pharmacological rescue experiments by acute application of SCN10a antagonists, thus indicating a potential therapeutic target in the treatment of PTHS. Development of new drugs for the treatment of psychiatric disorders, such as ASDs, has been hindered by a large translation gap between animal models and humans. We propose close this gap by determining if our current understanding of TCF4 function in animal models will translate into a human model of neuronal development. We have already established several induced pluripotent stem cell lines derived from PTHS patients and will increase our patient cohort through the generation of additional stem cell lines. Preliminary RNA sequencing data from human PTHS cortical neurons indicate an enrichment for genes associated with ion channels, axons, and synapses, that strongly correlates with our prior electrophysiology data in animal models. This proposed electrophysiological and transcriptional characterization will lead to the identification of novel downstream targets of TCF4 that will be pursued as potential therapeutic targets. If successful, our approach will help cross a large translational gap that currently exists between drug development in animal models and delivering effective treatments in humans.

Michael Nestor, Ph.D.
The Hussman Institute for Autism
Award Amount: $343,845
Disease Target: Autism Spectrum Disorder (ASD)

Establishing a 3D Based High-Content Screening Platform for Cellular / Phenotypes in Autism

Success in elucidating mechanisms behind Autism Spectrum Disorder (ASD) using human induced pluripotent stem cells (iPSCs) depends on cell culture techniques that approximate real brain circuitry and the ability to perform complex unbiased physiological analyses. This proposal bridges the gap between current 2D and 3D iPSC models of ASD to gain a better understanding of the etiology of ASD and to provide a robust platform for drug discovery/development. By using a novel 3D iPSC culture system called a serum free embryoid body (SFEB) that is highly replicable and recapitulates aspects of early cortical development, we will test the general hypothesis that excitatory and inhibitory (E/I) circuitry is dysfunctional in ASD. In this proposal, we create SFEBs from 4 genetically well characterized individuals and 4 unaffected controls. Using whole exome sequencing and transcriptomics, variants have been generally implicated in cell migration, vesicle cycling, and neuronal excitability. Preliminary data shows that across these 4 lines there is decreased network-level spiking activity and altered intracellular calcium ([Ca2+]in) transients. Application of alpha-latrotoxin, a black widow venom that causes a Ca2+-dependent presynaptic vesicle release, did not increase network spiking in ASD lines. Finally, we found significantly lower numbers of interneurons in SFEBs made from individuals with ASD. The activity of [Ca2+]in in development, particularly with respect to GABA neurotransmission, regulates E/I balance by affecting the proliferation and numbers of both excitatory and inhibitory neurons. The timing and amount of this shift also controls synaptic activity and the function of cortical networks. Our overall hypothesis is that aberrant E/I balance in individuals with ASD is caused in part by the dysregulated network and Ca2+ activity observed in iPSC-derived cortical neurons. In Aim 1, SFEBs will be studied using an unbiased high-content morphological analysis to determine the number and type of E/I inputs in developing cortical circuits in 3D. This will clarify the dynamics of E/I development in ASD. The role that dysregulated [Ca2+]in may have on the development of cortical circuits will be assayed using the genetically encoded Ca2+ indicator GCamp6. Circuit-wide electrophysiological recordings will be performed using our 3D culture system. We will isolate excitatory and inhibitory neurons by pharamacological methods, and/or by using an mCherry-VGAT promoter in SFEBs. In Aim 2, we develop an unbiased high-content genetics screen using CRISPR interference (CRISPRi) to measure the activity of [Ca2+]in and neural network activity in SFEBs. We will use CRISPRi in SFEBs made from control individuals to knock down the endogenous activity of the variants identified in our ASD cohort while measuring the electrophysiological profile and Ca2+ activity in 3D cortical circuits. In doing so, we will attempt to recapitulate phenotypes observed in Aim 1. The proposed work develops a system for the unbiased identification of mechanisms that affect E/I balance in ASD. Our 3D high-content screening platform will provide a valuable tool in pre-clinical lead development of interventions for ASD. To our knowledge, this is the first attempt to combine high-throughput imaging, electrophysiology, and CRISPR technology with 3D stem cell culture to study the etiology of ASD.

Linda Resar, M.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Colitis

Developing Stem Cell Technology for Tissue Repair and Goblet Cells for Ulcerative Colitis Patients

The primary goal of this project is to develop stem cell therapy to regenerate colon tissue for patients with ulcerative colitis (UC). The intestinal lining is among the most regenerative tissues, renewing itself every 3-5 days to protect the gut from bacterial pathogens and toxins. Intestinal stem cells (ISCs) located deep within the crypts are responsible for this remarkable capacity, although factors that govern their behavior are poorly understood. In recently published work (Xian et al, Nature Comm; 2017), we discovered that the High Mobility Group A (HMGA1) chromatin remodeling protein is a key epigenetic regulator that maintains ISC number and function within the small intestine. Using innovative mouse and organoid models with overexpression or deficiency of Hmga1, we found that Hmga1 promotes epithelial regeneration in the small intestines. Our preliminary unpublished studies suggest that Hmga1 also promotes stem cell function, tissue repair, and goblet cell differentiation in the colon. Goblet cells are important because they secrete mucous to protect the intestinal lining from pathogenic invasion and inflammation. Notably, there is a decrease in goblet cells and their protective mucous in the colon of patients with UC, which leads to excessive inflammation, tissue damage, and the acquisition of genomic lesions that increase the risk for colon cancer. Our preliminary results also suggest that enhancing HMGA1 expression improves colon-specific ISC function and tissue regeneration in a dextran sulfate sodium (DSS) model of UC. Thus, HMGA1 is a key developmental factor important in normal stem cell function and tissue repair. Because HMGA1 functions as a key regulator that modulates chromatin structure to induce stem cell gene expression, this led us to the hypothesis that stem cell therapy to increase HMGA1 activity will improve colon-specific ISC function, restore goblet cell number, and enhance tissue repair in UC. Specific Aims: To test this, we propose to harness our unique mouse and human organoid models with genetic manipulations in HMGA1 in the following Aims: 1) To define molecular mechanisms through which HMGA1 promotes ISC function, tissue repair, and goblet cell differentiation in colonic epithelium, 2) To determine whether Sendai virus can be used to engineer clinically safe, HMGA1-enriched, colon-specific organoids for personalized tissue regeneration in preclinical models of UC. These innovative studies will not only provide insight into colon ISC function and goblet cell differentiation, but could also uncover novel stem cell approaches to protect the intestinal lining, enhance tissue regeneration, and prevent precancerous and malignant lesions in UC. We will harness our stem cell therapies to benefit Maryland citizens with UC. Our approaches could also provide potential treatment for other intestinal diseases.

Piotr Walczak, M.D., Ph.D.
Johns Hopkins University
Award Amount: $345,000
Disease Target: Myelin

Inducing Immunotolerance of Myelinating Progenitor Cells Transplanted into the Brain of Immunocompetent Mice

Cell-based treatments are rapidly moving toward clinical application, including our proposed use of hGRPs, which recently received FDA clearance for treatment of ALS. Notwithstanding, one outstanding obstacle is the issue of transplant rejection for cell-based neurologic therapies. There is major uncertainty regarding the efficacy and safety of different immune suppression regimens, and even questions as to whether immunosuppression is needed at all. The prevailing approach in clinical trials has been pharmacological immuno-suppression administered for 2-6 months. While these protocols prevent rejection during the immunosuppression period, the treatments are toxic, interfere with differentiation of transplanted cells, and increase the risk of infections and cancer. In addition, detailed knowledge is lacking on the consequences of discontinuation of immunosuppression: whether it leads to reactivation of the immune response which could compromise the transplanted cells but importantly could also lead to catastrophic consequences to the recipients if large quantities of engrafted cells in the brain were suddenly rejected. Clearly, current immunosuppresion protocols are suboptimal and there is a critical unmet need to develop new strategies that are convenient, effective and safe. Learning from progress in the fields of vascularized composite allografts, and beta islet cell transplantation, we identified protocols that are promising for intracerebral stem cell grafts. Manipulation of co-stimulation signaling in T cells accomplished by blocking that signaling with monoclonal antibodies results in T cell anergy and prevents rejection. Co-stimulation blockade has been shown to induce a state of tolerance without compromising overall immune defenses and with least toxicity to grafted cells and to the host. A remarkable observation is that the costimulation blockade treatment delivered at the time of transplantation can induce long-lasting tolerance, bypassing the need for life-long immunosuppression. Here, we propose to perform a systematic assessment of both the efficacy and safety of this new immunoprotection strategy and compare it to current standard immunosuppression. First, we will perform a long-term efficacy study over 6 months to assess whether transplanted GRPs survive and restore myelin in shiverer mice immunosuppressed for 2 months with tacrolimus+sirolimus or in mice with immune tolerance induced by co-stimulation blockade. We will evaluate a critical safety aspect of how robust is the immunotolerance with either immunosuppression protocol. In our immunodeficient, hypomyelinated mouse model, we observed extensive engraftment of the graft with a large proportion of endogenous glia replaced. But this situation is extremely perilous if host versus graft response are reactivated at any time after transplantation. To study this phenomenon, we will transplant GRPs in dysmyelinated mice using these contrasting immunoprotection protocols, observe the animals for 6 months and then induce a clinically relevant neuroimmunologic challenge to the engrafted cells via a local traumatic brain injury or systemic administration of LPS. Our study will add fundamental new knowledge in cell-based therapy, including evaluating the efficacy and safety of prevailing immunosuppression protocols and validating a new tolerance-induction strategy that addresses many shortcomings of pharmacological immunosuppression.

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Award Amount: $345,000
Disease Target: Amyotrophic Lateral Sclerosis (ALS)

Highly Efficient Conversion of iPS Cells to Motor Neurons and Oligodendrocytes by Synthetic Modified mRNAs

Spinal cord motor neurons (MNs) and oligodendrocytes (OLs) derived from human induced pluripotent stem cells (iPSCs) provide unique cell resources for disease modeling, drug development and cell replacement therapy for neurological disorders, such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and various demyelinating diseases. Current differentiation methods for these cells are still slow, variable and inefficient. Here, we will develop a novel differentiation strategy based on delivering synthetic mRNAs coding transcription factor (TF) drivers of MN and OL conversion. Compared to traditional methods relying on chemical compounds or viruses, mRNA-induced differentiation is more specific, efficient and safe. With the continuous support from MSCRF, we have established a highly efficient strategy for generating iPSC-derived dopaminergic neurons by using synthetic mRNAs coding the Atoh1 TF with phosphosite modification (patented and licensed to Molecular Transfer, Inc.). Building upon this patented technology and our following research, we hypothesize that it is feasible to generate highly pure and functional mRNA-induced MNs (miMNs) and OLs (miOLs), by using mRNAs coding the Olig2 TF in combination with Ngn2 for MN and Sox10 for OL differentiation. Our preliminary results further support that defined phosphosite modification in these TFs will significantly enhance the efficiency of MN and OL conversion, through enhanced protein expression and/or more precise control of MN/OL fate commitment. The specific aims of this proposal are: (1) highly efficient generation of iPSC-derived MNs by using synthetic mRNAs coding modified Olig2 and Ngn2, and functional characterization of these miMNs; and (2) highly efficient generation of iPSC-derived OLs by using synthetic mRNAs coding modified Olig2 and Sox10, and functional characterization of these miOLs.

In this project, we will generate highly pure miMNs and miOLs from both normal and ALS-patient-derived iPSCs. We will further determine their similarity to bona fide MNs or OLs, through a panel of functional assays and global transcriptome analysis. It is innovative to use synthetic mRNAs to more rapidly and specifically active TF drivers of MN and OL conversion without relying on chemical compounds and viruses. Phosphosite modification of TF drivers is also an innovative strategy to significantly enhance the efficiency of mRNA-induced MN and OL differentiation. If successful, we will establish the first synthetic-mRNA-driven strategy for generating iPSC-derived MNs and OLs in high purity and with validated functions. These miMNs and miOLs will have wide applications in mechanistic studies, drug testing and cell replacement therapy for various neurological disorders. Our innovative strategy using synthetic mRNAs will also provide a solid foundation for generating hard-to-achieved lineage-specific progenies from human stem cells. We have been collaborating with Molecular Transfer, Inc., a biotech company in Maryland, to develop a mRNA-induced system for dopaminergic neuron differentiation. Through this collaboration, we will translate new inventions from this project into products, such as mRNA-induced MN and OL differentiation kit, and ready-to-use iPSC-derived MNs and OLs. Overall, inventions from this project will facilitate the production of MNs, OLs and likely other functional cells from human stem cells, and benefit biotechnology in Maryland.

Validation Grant Awards

Sharon Gerecht, Ph.D.
Johns Hopkins University
Award Amount: $230,000
Disease Target: Coronary Artery Diseases

Patient-Specific Small-Diameter Tissue Engineered Vascular Grafts

Surgical intervention using autologous arterial grafts is the most common corrective procedure for coronary artery diseases (CADs). However, many patients lack suitable donor tissue because of previous surgery or as a result of their underlying vascular diseases. Additionally, while use of synthetic vascular grafts for the Fontan and other procedures is common to treat pediatric congenital cardiovascular defects (CCD), it remains the leading source of morbidity and mortality associated with the procedure due to complications arising from the inability for synthetic grafts to grow as the child develops. Development of a material and a process that would enable the creation of a patient-specific fully functional, cellularized small-diameter tissue engineered vascular graft (sTEVG) would address the compelling clinical unmet need described above. We have recently generated a perfusable, cellularized sTEVG. Here we will combine our established stem cell differentiation and graft formation technologies for the formation of patient-specific functional sTEVGs to treat CADs and pediatric CCDs. Our team, including stem cell and vascular engineers, biomaterial scientists, and surgeons collaborating with Johns Hopkins Technology Ventures (JHTV), has developed a series of experiments to achieve the following milestones: (1) optimize sTEVG fabrication and (2) determine safety and efficacy of sTEVGs in vivo. Progress will be evaluated with the commercialization team at JHTV to ensure comprehensive and long-term development of the technology. Achieving these goals will prepare the technology toward scale up and pre-clinical testing.

Sunjay Kaushal, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Heart Regeneration

Neonatal Cardiac Stem Cells for Heart Regeneration

After a myocardial infarction, the mammalian heart undergoes only minimal levels of regeneration. While medical and surgical interventions are effective in limiting damage, these ischemic patients suffer from heart failure symptoms that grow worse over time, and can lead to disability and death. The most promising approaches to regenerate muscle and vasculature are cell-based therapies. A recent Phase I clinical trial of autologous (self-donated) cardiac stemcell therapy reported that left ventricular function improved an average of 12% in the year after treatment.1 However, even successful autologous cell therapies face obstacles to widespread adoption, including high expense, delays, and variable effectiveness. 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. We have shown that these c-kit+ CPCs are able to stimulate the regeneration of cardiac muscle and restore heart function in a rodent model of myocardial ischemia.2 This stem cell population can be developed into a therapy that incorporates streamlined manufacturing techniques, keeps costs down, and offers reproducible, well-defined benefits. These are key elements of a commercially-viable regenerative cell therapy. In this application, we propose to validate the safety and functional abilities of allogenic, neonatal, cloned, c-kit+ CPCs in a well-established swine acute myocardial infarction model. The study will be comprised of three groups of four pigs: high-dose CPC, low-dose CPC, and a control group. At study completion, we will have a powerful proof-of-concept validation that will serve as the template for the GLP study that will be a crucial step towards submitting an Investigational New Drug (IND) application for the use of neonatal-derived, cloned, c-kit+ CPCs in treating post-MI heart failure.

Chulan Kwon, M.S., Ph.D.
Johns Hopkins University
Award Amount: $230,000
Disease Target: Multiple

Developing Adult Cells from iPSCs

Induced Pluripotent Stem Cell (iPSC) technology is a standardized technology used to recapitulate relevant donor disease biology in the laboratory, a capability often referred to as "disease-in-a-dish," to gain new insights into disease mechanisms and to serve as a powerful pre-clinical approach for drug discovery, drug efficacy and drug cytotoxicity studies. However, a major limitation with the iPSC technology is that tissue cells generated from iPSCs in the laboratory remain fetal-like even after a year of maturation in a dish and that to date, no technology is available to mature iPSC-derived cells into functional adult cells. This has become a major and common impediment for their application in the iPSC field as most human diseases are age-related and appear when organs and cells have matured. Thus, developing technologies to generate mature iPSC-derived cells is in extremely high demand. By combining bioinformatics analyses and conserved early in-vivo cues, we have developed a technology to generate adult human tissue cells from iPSCs using rodent organs as bio incubators. This work has led to 239K article views and 1.15K social shares since its publication, reflecting the significance of this work in the field of iPSC-based therapeutics. The current proposal aims to scale and validate our technology in a large animal model, an essential step for commercialization. To do this, we have set specific goals with quantifiable milestones in collaboration with Johns Hopkins Technology Ventures: (1) To determine the scalability of iPSC-derived cardiomyocytes maturation in pig organs and (2) To determine the potential of the bio incubator for testing drugs to treat the adult-onset heart disease Arrhythmogenic Right Ventricular Cardiomyopathy. The MSCRF validation program will help us turn our technology into a novel drug discovery and validation technology for the pre-clinical market.

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Award Amount: $230,000
Disease Target: CNS Myelin Disorders

Lineage Reprogramming of Human Fibroblasts into Oligodendrocyte Progenitor Cells

Cell transplantation therapy using oligodendrocyte progenitor cells (OPC) and or functional oligodendrocytes (OL) for treating dysmyelinating and demyelinating CNS disorder is largely limited by the inability to obtain a pure self renewal OPCs. Several approaches have been established to derive OPCs from the fetal brain or human embryonic stem cells (hESCs) as well as from human induced pluripotent cells (hiPSCs). However, these approaches are time-consuming, and inefficient, generating heterogeneous cells and tumorigenic potential, and thus have limitations for regenerative medicine. Establishing protocols which can directly convert (i.e., reprogram) somatic cells such as fibroblasts into induced OPCs(iOPCs) and OLs (iOLs) are urgently needed. Recently, we have established an efficient hiPSCs-derived iOPCs protocol using nanoparticle delivery transcription factor mRNA cocktail combined with the oligodendrocyte specification signals. Moreover, using this protocol with some modifications, iOPCs can be directly generated from human fibroblasts. It is now important to establish more precisely lineage reprogramming protocol to generate OPCs from human fibroblasts. Using established lineage reprogramming protocol, in Aim # 1 we will generate iOPCs derived from human fibroblasts; and we will determine the iOPC transcriptome using RNS-seq; in Aim # 2, we will extensively determine the functionality and safety of iOPCs in vivo.Successful completion of this proposal 1) will for the first time enable us to generate iOPCs and iOLs without genetic manipulation from human somatic fibroblasts in a clinically applicable timeframe. This work will generate a patentable procedure for the generation of a cell source that can be used for iOPCs-based transplantation, central nervous system(CNS) demyelinating disorder modeling and drug screening for therapeutic purposes; 2) will provide safety data for future clinical trials with human fibroblasts-derived iOPCs; 3) will provide a cellular platform to study molecular mechanisms underlying OPC lineage reprogramming and CNS demyelination or dysmyelination.

Elias Zambidis, M.D., Ph.D.
Johns Hopkins University
Award Amount: $230,000
Disease Target: Coronary Artery Diseases

MoroPLUR: A Defined Feeder-Free Medium for Enhancing Functionality of Human Pluripotent Stem Cells

We recently reported that stable transgene-independent mouse ESC (mESC)-like naïve human pluripotent stem cells (N-hPSC) with decreased lineage-primed gene expression and improved differentiation potency could be derived by chemically reverting conventional, primed hPSC with LIF (leukemia inhibitory factor) and only WNT, MEK/ERK, and tankyrase inhibition (LIF-3i) (Zimmerlin et al, 2016) http://dev.biologists.org/content/143/23/4368. A broad repertoire of conventional hESC and transgene-independent hiPSC lines were maintained in a naïve-like state in standard feeder-dependent (MEF) conditions with only LIF-3i. LIF-3i/MEF N-hPSC cultures attained defining characteristics of mESC including naïve-specific transcriptional and epigenetic features, and a high clonal self-renewal that was independent of MEK-ERK signaling, but dependent on JAK-STAT3 and BMP4 signaling. Tankyrase inhibition promoted stable acquisition of a human preimplantation ICM-like ground state via WNT signaling modulation. Most importantly, in contrast to other recently reported methods of chemical naïve reversion, a broad repertoire of LIF-3i-reverted N-hPSC retained normal karyotypes and epigenomic imprints within the context of a globally hypomethylated CpG methylome.

Our Objective is to validate and commercialize our tankyrase inhibitor-based naïve-reverting culture system to a defined, feeder-free (FF), small molecule-based medium called MoroPLUR, that efficiently and stably reverts hPSC to a naïve-like state with augmented differentiation potency and increased functionality. Our Specific Aims will validate the efficacy of FF MoroPLUR reversion medium in multi-lineage directed differentiation experiments, as well as with a novel in vivo interspecies murine-human fetal chimera pluripotency assay recently developed in the Zambidis lab. Our Central Hypothesis is that FF MoroPLUR-reverted N-hPSC with decreased lineage bias and increased functional pluripotency will not only increase the differentiation potency of conventional hPSC, but also allow future generation of specific transplantable human lineages in interspecific blastocyst complementation chimeras. We propose that making FF MoroPLUR widely accessible through commercialization will dramatically change the landscape of regenerative medicine and experimental human disease modeling.

Commercialization Grant Awards

Hai-Quan Mao, Ph.D.
LifeSprout, LLC.
Award Amount: $300,000
Disease Target: Multiple

Delivery of MSCs to Enhance the Replacement and Regeneration of Soft Tissue

LifeSprout has recently developed a novel injectable and bioresorbable nanofiber-hydrogel composite known as the Regenerative Tissue Matrix (RTM) for the replacement and reconstruction of soft tissue defects. The novel RTM delivery matrix holds promise to improve clinical outcomes for reconstruction of soft tissue loss following cancer surgery, trauma, and birth defects which affect more than half a million patients each year. As demonstrated in multiple in vivo preclinical models, the RTM tissue filler provides immediate volumization and soft tissue feel by simulating the viscoelastic property of natural soft tissue, and more importantly promotes extensive revascularization at the tissue repair site. The overall objective of LifeSprout's Maryland Stem Cell Research Fund (MSCRF) Commercialization Grant is to extend the utility of the current RTM composite by: (1) optimizing the matrix for the delivery of mesenchymal stem cells (MSCs) and (2) validating the key product features and competitive advantages of LifeSprout's novel MSC delivery matrix compared to the market benchmark for large volume soft tissue reconstruction. The incorporation of stem cells into LifeSprout's injectable composite offers a potential breakthrough technology in reconstructive medicine. With the new MSC-seeded RTM, reconstructive surgeons could for the first time offer patients a minimally invasive, cost-efficient, and regenerative restoration for medium and large three-dimensional soft tissue losses.

Ha Nam Nguyen , Ph.D.
3Dnamics Inc.
Award Amount: $300,00
Disease Target: Alzheimer's Disease

Engineering Human Pluripotent Stem Cell-Derived Brain Organoids for Drug Screening and Toxicity Testing

After a myocardial infarction, the mammalian heart undergoes only minimal levels of regeneration. While medical and surgical interventions are effective in limiting damage, these ischemic patients suffer from heart failure symptoms that grow worse over time, and can lead to disability and death. The most promising approaches to regenerate muscle and vasculature are cell-based therapies. A recent Phase I clinical trial of autologous (self-donated) cardiac stemcell therapy reported that left ventricular function improved an average of 12% in the year after treatment.1 However, even successful autologous cell therapies face obstacles to widespread adoption, including high expense, delays, and variable effectiveness. 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. We have shown that these c-kit+ CPCs are able to stimulate the regeneration of cardiac muscle and restore heart function in a rodent model of myocardial ischemia.2 This stem cell population can be developed into a therapy that incorporates streamlined manufacturing techniques, keeps costs down, and offers reproducible, well-defined benefits. These are key elements of a commercially-viable regenerative cell therapy. In this application, we propose to validate the safety and functional abilities of allogenic, neonatal, cloned, c-kit+ CPCs in a well-established swine acute myocardial infarction model. The study will be comprised of three groups of four pigs: high-dose CPC, low-dose CPC, and a control group. At study completion, we will have a powerful proof-of-concept validation that will serve as the template for the GLP study that will be a crucial step towards submitting an Investigational New Drug (IND) application for the use of neonatal-derived, cloned, c-kit+ CPCs in treating post-MI heart failure.

Madhusudan Peshwa, Ph.D.
MaxCyte, Inc.
Award Amount: $300,000
Disease Target: Sickle Cell Disease (SCD)

Translational Development of Gene-Corrected Hematopoietic Stem Cells as Treatment for Sickle Cell Disease (SCD)

The quest to find novel therapeutics for mental and neurological disorders has been hindered by the lack of access to live human brain samples and relevant experimental models. Currently, roughly 0.1% of drugs that show promise in preclinical testing make it to Phase I clinical trials in humans, and 90% of those drugs go on to fail FDA approval. This is largely because animal and two-dimensional cell culture models are not accurate enough predictors of how drugs will work in humans. Recent progress in three-dimensional (3D) tissue engineering offers a promising platform that may be the key in accelerating and improving current drug developmental trend. These 3D tissues or organoids are differentiated from human pluripotent stem cells (PSCs) to resemble specific parts of the human brain, which include architecture composition and physiology. 3D brain organoids enable significant advantages in key areas of drug development: toxicity testing, drug validation and screening, and disease modeling. Due to the high demand of PSC-derived brain organoids, 3Dnamics proposes to scale-up and to commercialize the 3D brain organoid system to perform high-throughput drug screening for efficacy and safety. An optimized and consistent 3D human cell-based model that can much more accurately predict human drug responses will significantly increase success rate of developing treatments for brain-related disorders, such as glioblastoma and Alzheimer's disease. To date, we have partnered with several pharmaceutical companies and academic laboratories to use our brain organoid system to develop treatments for Zika virus infection and Parkinson's disease. In addition, we have obtained seed funding from Camden Partners. The funding from MSCRF will accelerate our products to the mass market.

Jonathan Rowley, Ph.D.
RoosterBio, Inc.
Award Amount: $300,000
Disease Target: Multiple

Closed Systems Enabling Commercially-Viable Stem Cell Manufacturing

A critical bottleneck impeding the growth of Regenerative Medicine is the availability of cGMP-compliant stem cells for clinical development. Hence there is an immediate need to develop platform systems that simplify the production of cGMP-compliant stem cells and radically reduce clinical translation timelines. A need for affordable production of cells, including human Mesenchymal Stem Cells (hMSC), in closed scalable systems has already been identified as a "Current Challenge" in the Technology Roadmap established by the NIST-funded Cell Manufacturing Consortium. This proposal specifically focuses on the development and qualification of a functionally closed version of the working cell banks for use in scalable stem cell manufacturing. To achieve this goal, it is necessary to 1) develop and fully document the production chain of closed system hMSC working cell banks and 2) complete and document comparability studies of the standard vial-based open system vs the innovative closed system bagged hMSCs. Within 6-12 months of completing this proposal, RoosterBio will bring to market the novel closed system hMSC working banks, labeled 'For Further Manufacturing', which are expected to be seed stocks for cGMP manufacturing. This product will shave several years and millions of dollars off the typical Regenerative Medicine product development timelines. While the technical risks are limited, the market risk of a technology too early for market uptake will be addressed by a multi-year shelf life that allows for elastic production. These products will generate tens of millions of dollars in revenue for RoosterBio over the next 3-5 years, generating significant job growth, and driving Maryland's bioeconomy. By providing cGMP hMSCs for the Regenerative Medicine field, thought to now be entering into the inflection phase of the Gartner Curve, RoosterBio is well poised to be a leading manufacturer for Regenerative Medicine for years to come.

William Rust, Ph.D.
Seraxis Inc.
Award Amount: $298,960
Disease Target: Diabetes

Long-Term Function of Stem Cell Grafts for Insulin-Dependent Diabetes

Major progress has been made towards the development of a stem cell-derived cure for insulin-dependent diabetes. Stem cells can generate an unlimited supply of insulin-producing cells, and immune-protective capsules enable the cells to provide long-term control of diabetes in animal models. For this therapy to be practical for the millions of patients in need, an implant strategy that enables engraftment of the capsules, is retrievable, and is safe must be proven to the standards of regulatory authorities. This proposal aims to evaluate the interaction between the stem cell therapy implant and the host tissue on the long-term safety and efficacy of Seraxis cell therapy cure for diabetes. The proposed project involves the proprietary iPS cell line, SR1423, which was developed from a human pancreas and came from a consented anonymized deceased adult donor. His organs, including the pancreas, were re-covered by the California Transplant Donor Network in full compliance with U.S. ethics laws, after the completion of a donor medical and social history questionnaire. To confirm eligibility, the donor was screened for the presence of transmissible pathogens, according to 21 C.F.R. Part 1271 regulation. The project also involves the use of a diabetic rat model. Type 1 diabetes afflicts 1.25 million American, whose life expectancy is often shortened by a broad spectrum of co-morbidities. Despite the availability of in vitro assays to assess the production of insulin by cells, there is presently no substitute to the use of animals that could accurately assess the reversal of diabetes: a cure to diabetes not only requires proper vascularization of the implanted insulin-producing cells, but the cells also need to survive the host immune attack, which can only be assessed in a diabetes animal model. All the animal work will be contracted out to BioQual Inc., whose facilities are AAALAC accredited and OLAW assured. The lowest possible number of animals will be used. Prior to study initiation, the protocol will be submitted for IACUC approval. During the study, all measures will be taken to minimize pain and avoid animal distress.

Chengkang Zhang, Ph.D.
Propagenix Inc.
Award Amount: $300,000
Disease Target: Cystic Fibrosis (CF)

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

Basal stem cells located in stratified and pseudostratified epithelia throughout the body drive both normal epithelial homeostasis and tissue repair after injury. Despite their infinite self-renewal capability in vivo, these cells can only be expanded for a few passages in vitro before they succumb to irreversible stress-associated cell growth arrest. Furthermore, late-passage cells do not retain full differentiation functionality using current protocols and culture conditions. To address these limitations, we have developed EpiX™: a serum- and feeder cells-free cell culture technology that supports unprecedented expansion of tissue-resident basal stem cells in skin, airway, cornea, and other epithelia. These tissue-resident basal cells can be expanded over trillion-fold (10e12) in standard 2D culture. They also readily differentiate into lineage-specific mature epithelial cells phenotypes consistent with their tissue origins upon exiting the expansion condition. This application focuses on 1) final optimization of expansion and differentiation media and protocols for human airway basal cells; 2) demonstration the utility of this technology for personalized medicine applications using patient-derived cells; and 3) medium production scale-up from bench scale to lot sizes of 25 to 100 liters. This will provide support to move forward with full commercialization. Initial products will consist of expansion and differentiation media for end users engaged in basic research on normal and diseased lung biology. Follow-on products will likely include ready-to-use differentiated cells and tissues for drug discovery and toxicology evaluation. Because EpiX™ technology also supports efficient single cell cloning and provides sufficient proliferative runway for genome editing, we anticipate that it will also have commercial value in cell therapy for many respiratory diseases, e.g., cystic fibrosis (CF), severe asthma, and COPD. To that end, we have also developed formulations of EpiX™ medium lacking animal-derived components to provide manufacturing- and regulatory-friendly solutions required of cell replacement therapies.

Post Doctoral Fellowship Grant Awards

Qin Bian, Ph.D.
Johns Hopkins University
Mentor: Patrick Cahan, Ph.D.
Award Amount: $130,000
Disease Target: Osteoarthritis

Direct Specification of Articular Chondrocytes from iPSC-Derived Lateral Plate Mesoderm

Osteoarthritis is a common disease in which the degradation of articular cartilage results in bone damage, pain, and reduced mobility. Current treatments focus on symptomatic relief through pain-relief medications or joint replacement. However, neither of these interventions restore physiological articular cartilage function. Articular cartilage is generated and maintained by specialized cells termed articular chondrocytes (ACs). Injury to ACs is a key event in the onset of OA due to their limited self-repair capacity. Two sources of engineered chondrocytes have so far proven disappointing. First, chondrocytes differentiated from mesenchymal stem cells undergo hypertrophy, reminiscent of the natural course of endochondral ossification that occurs when non-articular chondrocytes give way to bone during development. Second, autologous ACs must be expanded in vitro and doing so results in their dedifferentiation to fibroblast-like state that fails to adequately regenerate damaged cartilage. Human pluripotent stem cells (hPSCs) represent a potentially unlimited source of autologous cells to treat and model degenerative diseases such as OA. However, there are relatively few directed differentiation protocols purporting to generate PSC-AC. Moreover, the molecular fidelity of the current PSC-AC has not been comprehensively assessed. To address these issues, I propose to :

Specific Aim 1. Improve molecular and functional characteristics of PSC-AC through inducible ectopic expression of superficial specific transcription factors (TFs) in PSC-derived lateral plate mesoderm (LPM).

Specific Aim 2. Augment a network biology based computational platform, CellNet, so that it can assess and suggest improvements for engineering PSC-AC.

To achieve the goal, first: I will use published PSC-LPM differentiation protocol and then develop downstream LPM-AC differentiation protocol by overexpression of superficial specific TFs in LPM. These TFs will be determined based on our single cell RNA-Seq data from murine primary ACs. The fidelity of LPM derived ACs will be assessed by in vitro AC specific marker staining and in vivo transplantation at functional level and by CellNet at molecular level. Next, curate articular chondrocyte gene expression data from two publicly available datasets, run the newly curated AC data through our data pre-processing, gene regulatory network (GRN) reconstruction, and training pipeline in order to update CellNet by incorporating tissue-specific AC data. Then, using re-trained CellNet, we will assess the fidelity of our engineered cells by generating classification and GRN status scores.The overall goal of this proposal is two-fold. First, we will engineer human articular chondrocytes from hPSC, which can subsequently be used for modeling OA, drug screening, and ultimately for regenerative medicine. The second goal of this proposal is to deepen my training in the use of human iPSC and in computational biology.

Dongwon Kim, Ph.D.
Johns Hopkins University
Mentor: Luis Garza, Ph.D.
Award Amount: $130,000
Disease Target: Skin Disease

Postdoctoral Training Towards Independence: Testing of Skin Stem Cells to Modify Skin Identity

A total of 3,053 amputations were performed in Maryland hospitals in 2013. Amputation rates increased from 2011 (2,759) to 2013 (3,053) with improvements in body armor. Tremendous advances are also being made in prosthetics. However, even in the best conditions the use of prosthetics is still dramatically limited by discomfort, pain and skin-breakdown at the stump site. Skin can be divided into hairless volar (palmo-plantar) and hairy non-volar type. In particular, volar epidermis is markedly thicker than skin at other locations and uniquely expresses KERATIN 9 (KRT9) which itself makes it more friction- , pressure- and irritant-resistant through its unique keratin imparted strength. If the skin at the stump site is permanently converted into thick volar type skin with KRT9 then the use of prosthetics will be dramatically enhanced. In this grant, we propose to convert skin identity from non-volar to volar after the injection of volar fibroblast stem cells. We and others have shown that volar fibroblasts have the ability to increase epidermal thickness in 3-D and induce ectopic KRT9 in non-volar human epidermal stem cells (keratinocytes) in both 2-D and 3-D cocultures. Given that KRT9 itself provides structural strength, and unique to volar skin, it is an ideal read-out. We are currently testing the ability of fibroblasts stem cells to convert the skin identity in human volunteers partly through a grant from MSCRF. After confirming purity and sterility, we have injected these autologous cells into human subjects in paired locations in the buttocks skin, along with a vehicle control. In early preliminary results, we find that buttocks skin develops a thicker, denser collagen network leading to heavier skin. The epidermis is slightly thickened and now expresses ectopic KRT9. Our results show that volar fibroblasts stem cells in human subjects have the unique ability to reprogram nonvolar epidermal stem cells to create ectopic volar skin. The problem is that KRT9 is only partially induced, and not to high levels seen in the volar skin. Also the epidermis is only mildly increased in thickness, and not to the degree seen in volar skin. For those reasons, further improvements are highly required to enhance the efficacy and activity of ectopic volar fibroblasts in the regulation of epidermal stem cells, before wide-spread testing on the vulnerable population of amputees. In our recent paper, we have shown how damage induces regeneration. Mild skin damage will enhance activity of ectopic volar fibroblasts stem cells and induction of volar features in human subjects. To investigate this, this grant will employ autologous fibroblasts stem cells in skin recipient site with or without light curettage (scraping). Eventually injected skin area will be removed for histology and molecular studies to determine whether light curettage enhances the acquisition of volar features. The ectopic gene expression induced by volar cells will be verified through deep sequencing technology (RNA-sequencing) to optimize and maximize the potency of volar cells for manufacturing. The permanent change of skin at the stump site holds the potential to radically enhance the lives of our wounded Maryland residents and more significantly to help the more than 1.7 million (1out of every 200) people in the US who have had limb amputations. The success of this grant will contribute to develop both basic and translational research to advance the knowledge andtechnology in the stem cell therapy in human subjects.

Josephine Lembong, Ph.D.
University of Maryland - College Park
Mentor: John Fisher, Ph.D.
Award Amount: $130,000
Disease Target: Vascular / Bone Disease

Stem Cell Expansion and Differentiation in Bioreactors via Coupling of Substrate Curvature and Shear Stress

The demand for generation of large numbers of primary stem cells has become ubiquitous in many basic and translational stem cell research problems. Primary stem cells are however operationally inaccessible to most researchers because they are rare, expensive, and they rapidly lose their self-renewal capacity during in vitro culture. Our goal is to create an improved ex vivo culture of stem cells that would enhance the production of large numbers of phenotypically desirable stem cells to facilitate stem cell research and potential regenerative medicine therapies in the future. To do this, we propose to develop a dynamic perfusion bioreactors, which have been shown to be beneficial for cell proliferation and differentiation due to the control over environmental parameters, e.g. shear stress imposed on the cell surface, media flow, and oxygen distribution. Three-dimensional (3D) printing techniques allow us to fabricate flow chambers for such bioreactor applications, with precise control of geometry and hence flow. Particularly we choose to study in details the effect of substrate curvature, surface area, and shear stress on the growth and differentiation of human mesenchymal stem cells (hMSCs) in our flow chambers. Using our understanding of the phenomenology of the coupling between substrate curvature, flow, and cellular behavior in the bioreactors on a cellular level, we aim to design a more effective bioreactor for hMSC expansion. In particular, we seek a bioreactor geometrical design for co-culture of hMSCs and endothelial cells (ECs) that will optimize the osteogenic and angiogenic potential of the cells. Such knowledge will be applicable in designing grafts with such spatial control that allows prevascularization of large tissue engineered bone constructs which is crucial prior to patient implantation. One of the biggest challenges limiting wider use of stem cells in regenerative medicine is obtaining sufficient number of cells while at the same time maintaining the self-renewal capacity of the stem cells. To help address this issue, we propose to develop a 3D printed, tubular flow chamber / perfusion bioreactor for the culture of human mesenchymal stem cells. Such development stems from our knowledge of cellular behavior inside these bioreactors that is acquired by studying the interactions between cells, substrates, and flow. We propose to develop these bioreactors for use in the tissue engineering and stem cell markets. A 2012 review of the tissue engineering and stem cell commercial markets indicates “a path pointing toward continued success” [67]. From 2008 to 2011, there was nearly a three-fold growth in sales to $3.6 billion, while the number of companies increased two-fold to over 200. Leading sectors of the filed include orthopedic applications, wound healing, and stem cell banking, all of which would benefit from the work described in this proposal. Finally, over 80% of the preclinical, clinical, and service stage products under development use a cell-based tissue engineering strategy, and thus may benefit from the proposed work.

Joseph Mertz, Ph.D.
Johns Hopkins University
Mentor: Donald Zack, Ph.D.
Award Amount: $130,000
Disease Target: Glaucoma

Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-Derived Retinal Ganglion Cell

Several clinically important neurodegenerative diseases (e.g. glaucoma) are characterized by progressive loss of retinal ganglion cells (RGCs). Although reasonable therapies exist, they are not always effective, and there are no available neuroprotective agents to halt vision loss. Our goal is to increase our understanding of RGC neuroprotective signaling networks active during axonal injury using a multi-tiered systems-based approach in a highly disease-relevant stem cell based model. We seek to characterize and quantify the whole proteome and phosphoproteome in human embryonic stem cell-derived RGCs (hscRGCs). To further our understanding of signaling in RGC cell death, we will also perform these comprehensive examinations in a pharmacological axon injury and cell death model as well as more precisely examine proteins and phosphorylation events within the DLK/JNK/MAPK pathway using targeted selected reaction monitoring (SRM) approaches. We expect our results to greatly increase our understanding of the signaling networks, primarily centered on DLK, that bring about RGC cell death, and hopefully help inform the search for neuroprotective treatment strategies for glaucoma and other optic nerve diseases. The research strategy, training, and mentorship involved in this proposal are also designed to prepare me for an independent scientific career focused on systems-based analysis of stem cell-derived and other highly relevant models. This proposal seeks to thoroughly characterize the whole proteome and phosphoproteome in human embryonic stem cell derived retinal ganglion cells (RGCs), with added focus within a pharmacological axon injury model. Furthermore, it seeks to develop new selected reaction monitoring (SRM) proteomics assays targeting the members of the Dual Leucine Zipper Kinase (DLK) – c-Jun N-terminal kinase (JNK) – Mitogen activated protein kinase (MAPK, DLK/JNK/MAPK) pathway and implement them in this novel cell model. The anticipated results will increase our understanding of the overall signaling networks leading to RGC cell death, the involvement of DLK/JNK/MAPK signaling in particular, and hopefully accelerate the development of therapeutic neuroprotective strategies to prevent or halt vision loss in patients with glaucoma and other forms of optic nerve disease.

Fahimeh Mirakhori, Ph.D.
Johns Hopkins University
Mentor: Gabsang Lee, Ph.D.
Award Amount: $130,000
Disease Target: Neuronal / Myelination Diseases

Establishment of Human Schwann Cell In-Vitro Myelination

Schwann cell (SCs- main glia cells in peripheral nervous system) myelination is a complex dynamic developmental and post-developmental process required for nerve development and function. Most of our today's knowledge of these procedures have stemmed from snapshot views of in vivo studies which limit the longitudinal study at the cellular level. In this regard, in vitro myelination has mostly been studied with co-cultures of rodent dorsal root ganglion and SCs. However, having a human in vitro model can be a crucial platform not only to recapitulates human myelin development, disease and drug discoveries, but also to bridge the gap between models and man. Despite its necessity, no in vitro myelination analogous is available for human and no one has ever succeeded in developing such system. Here, we proposed a novel all human functional cell-based model to recreate myelin in a dish, can be ideal for screening myelin promoting compounds. We hope this will accelerate discovery of new therapeutics or refinement for de/dysmyelinating disorders such as multiple sclerosis, Guillain-Barré syndrome, Charcot-Marie-Tooth disease, diabetic neuropathy and spinal cord or peripheral nervous system injuries and infectious viruses, prior to the initiation of human clinical trials. We developed a 12-week protocol for SC specification and initiation of myelination by testing dozens of chemical compounds. We will use this system to investigate the following aims:

Specific Aim 1. To promote human SC in vitro myelination by using chemically defined factors.

Specific Aim 2. To characterize human functional SC population.

We will optimize current SC protocol for more robust myelination, and characterize theputative SC populations with numbers of functional analysis. Using fluorescent cell sorting (FACS), we isolated different putative SC populations for global transcriptome analysis. Our proposed studies will be beneficial to model various genetic and traumatic europathies and infectious diseases (e.g. Zika virus for Guillain-Barré syndrome-like symptom), not to mention the future cell replacement approaches.

Nikhil Panicker, Ph.D.
Johns Hopkins University
Mentor: Luis Garza, Ph.D.
Award Amount: $130,000
Disease Target: Parkinson's / Neurodegenerative Diseases

Activation of the NLRP3 Inflammasome in Human Dopamine Neurons as a Consequence of Parkin Dysfunction

Parkinson's Disease (PD) is a chronic, progressive, neurodegenerative disorder that affects over a million people within the U.S. It is characterized by progressively worsening motor deficits such as resting tremor, bradykinesia (slowed movement), rigidity, postural instability, and in later stages, cognitive defects. Underlying the clinical symptoms of PD is the degeneration of dopamine (DA) neurons located in the pars compacta of the substantia nigra (SNpc) (1). Inhibition of the E-3 ubiquitin ligase Parkin is central to PD progression; more than 100 loss-of function familial PD causing mutations in the gene have been identified, and the protein has been demonstrated to be inhibited under sporadic conditions. Current studies are underway to elucidate how Parkin dysfunction mediates PDassociated neurodegeneration. The current consensus is that Parkin contributes to mitochondrial quality control, and that a build-up of its ubiquitination substrates following its inhibition/mutation and contributes to PD progression. Despite recent advances that have helped us understand the factors that contribute to PD development, no current drug or treatment strategy can successfully halt or prevent 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 identified because the underlying physiology of these systems differs substantially from that of human DA neurons. To address this issue, we have generated human embryonic stem cell (hES) differentiated DA neurons (hDA neurons). We have 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. Preliminary data also indicates that NLRP3 itself might be a Parkin substrate. The NLRP3 inflammasome is a multimeric complex of proteins that has previously been shown to mediate hyperinflammation and pyroptosis (cell death with inflammation) in immune cells, but not in neurons. The proposed project seeks to build upon these novel findings and aims to elucidate a) Whether NLRP3 inflammasome activation occurs in neurons downstream of Parkin inactivation, b) If increased NLRP3 expression upon Parkin inactivation/deletion occurs because NLRP3 is a bona fide Parkin substrate in hDA neurons, c) Whether NLRP3 inflammasome activation contributes to neuropathological/mitochondrial deficits seen in Parkin KO hDA neurons. Our proposed signaling mechanism is summarized in Scheme 1. This project seeks to address the drawbacks of using traditional PD model systems and elucidate a human DA neuron specific signaling pathway that contributes to the death of neurons in PD. To accomplish this, we will use hDA neurons as our primary model system. Drug targets identified in this manner may have substantial potential as PD therapies.

Marco Santoro, Ph.D.
University of Maryland - College Park
Mentor: John Fisher, Ph.D.
Award Amount: $130,000
Disease Target: Vascular Disease / Angiogenesis

Development of Tissue-Engineered Vascularized Scaffolds via 3D Printing of Endothelial/Stem Cells

Tissue damage and loss of organ function is a common outcome of several medical conditions and traumatic accidents. Organs transplantation is the standard treatment for these conditions as the human body is unable to regenerate large tissues. Unfortunately, the shortage of organs available through donations and the growing list of recipients limit the number of patients that can benefit from organ transplantation. Tissue engineering (TE) holds the promise to meet this urgent need by developing implantable scaffolds that can initially serve as tissue replacement and subsequently foster tissue regeneration. Most tissues and organs require an extensive vasculature to provide cells with oxygen and nutrients and conventional TE approaches rely on the regenerative ability of the body to form such vascular network throughout the scaffold. Unfortunately, sprouting of pre-existing blood vessels is slow and results in limited cell viability within large scaffolds. Hence, scaffold vascularization has been recognized as one of the key challenges in TE and the major obstacle toward the regeneration of viable tissues and organs of clinically relevant size. In this proposal we seek to address this critical challenge by combining the use of endothelial/stem cell cocultures with the versatility of 3D printing (3DP) technology to fabricate scaffolds hosting an interconnected vasculature template. Several studies investigated the coculture of endothelial cells (ECs) and mesenchymal stem cells (MSCs) in 3D systems under flow perfusion to form perfusable blood vessels. Yet, there is little understanding on how MSC presence and shear stress cooperatively drive the formation of new vasculature and how these findings can guide the design and fabrication of vascularized scaffolds. Toward this end, in Aim 1 ECs and MSCs will be cocultured in different ratios in gelatin methacrylate (GelMA) scaffolds fabricated via 3DP and exposed to flow perfusion. As several key pathways involved in angiogenesis/arteriogenesis can be regulated by both EC:MSC crosstalk and by flow-derived mechanical stimulation, angiogenic response and stem cell differentiation will be evaluated in both cell types and correlated to the culture conditions. The mechanistic knowledge governing angiogenesis in 3DP cocultures of ECs/MSCs will serve as design criterion in Specific Aim 2 for 3DP interconnected EC:MSC-laden GelMA networks within polycaprolactone (PCL) networks. 3DP technology offers the possibility to replicate vessel morphology and to deposit specific cells in a spatial pattern similar to that of native vasculature. In this aim, we will evaluate the effect of different fabrication parameters (fiber spacing, size) on the successful formation of a homogeneous vascular network by EC:MSC-laden GelMA fibers within a PCL scaffold with the desired mechanical properties for in vivo implantation.
Multiphase PCL/GelMA scaffolds designed and optimized in Aim 2 will be then tested and validated in vivo in an established animal model in Aim 3. Different experimental groups will be tested in order to discern the relevance (and necessity) of each scaffold component (ECs, MSCs, flow perfusion preconditioning, and PCL network presence) toward the formation of a functional and stable vasculature in vivo. Overall, the proposed system would address one of the key challenges in TE and regenerative medicine. Ultimately, the proposed research has the potential to advance the clinical treatment of virtually all those conditions where a severetissue defect is present.

Congshan Sun, Ph.D.
Johns Hopkins University
Mentor: Gabsang Lee, Ph.D.
Award Amount: $130,000
Disease Target: Duchenne Muscular Dystrophy (DMD)

hiPSC Based Compound Screening for Treatment of Duchenne Muscular Dystrophy

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 is only one approved drug available targeting 13% of the DMD patients, and there is a great need for more treatment options. Recent results from our laboratory demonstrate a fusion defect in multiple lines of human induced pluripotent stem cell (hiPSC)-derived DMD patient myoblasts. This can be used as a phenotype to screen chemical compounds for DMD therapy. We made a start by performing medium-scale chemical compound screen, composed of 1524 compounds from Johns Hopkins Clinical Compound Library, on myoblasts derived from one DMD hiPSC line. Myotube formation and associated protein expression were used as parameters to select hit compounds. We selected 19 hit compounds from the primary screening based on the above two parameters. Here, we propose to carry out a tiered screening process to validate and optimize the concentration of positive hits on multiple DMD-iPSC lines carrying different genetic mutations. Based on the positive hits, we also will seek to link additional pathways to DMD pathogenesis. Finally, with the result we can test newly identified compounds in vivo using the cell transplantation model. This project will greatly promote hiPSC application in muscular dystrophy disease modeling and DMD therapy discovery. Also this project is highly translational as the resulting compounds can be further introduced to clinical trial facilitating stem cell therapy. For personal development, I have been working as a postdoc for three years so far. As an enthusiastic young stem cell researcher, I am dedicated to stem cell therapy. I enjoy research and hope I will become an independent researcher one day. While conducting the stem cell research in Johns Hopkins, the MSCRF would help me to start with being independent doing this project and to go further in my academic career.

Aline Thomas, Ph.D.
Johns Hopkins University
Mentor: Jeff WM Bulte, Ph.D.
Award Amount: $130,000
Disease Target: Multiple Sclerosis (MS)

Development of Stem Cell Therapies for Multiple Sclerosis using Non-Invasive Biomarkers

Multiple sclerosis (MS) is an autoimmune disorder that targets myelin, the insulating layer that controls nerve function and survival. Worldwide, 1.4-2.0% of people are expected to be affected by [1], which is characterized by progressive paralysis that results in healthcare costs of over 70,000 USD per patient per annum [2-4]. Current treatments aim to slow the rate of paralysis using nontargeting immunosuppressive drugs [2, 5, 6] that hinder the entire immune system. However, many of these drugs are toxic for the patient [7]. Recently, the transplantation of stem cells has been explored to reduce the paralysis and other disease symptoms characteristic of MS as a natural alternative to toxic immunosuppressant drugs.This proposal aims to develop stem cell transplantation-based therapies to reduce the debilitation caused by the autoimmune disease experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Mesenchymal stem cells and glial progenitor cells have both been shown to reduce the autoimmune response in multiple sclerosis. Additionally, both stem cells have been found to protect glutamate-sensitive cells, neurons and oligodendrocytes, from excitotoxicity. 19-fluorine magnetic resonance imaging and bioluminescence imaging will reveal how long the stem cells survive in vivo. Variable-delay multi-pulse chemical exchange saturation transfer magnetic resonance imaging, a novel glutamate-sensitive imaging method, will reveal whether these stem cells can also protect the central nervous system from glutamate toxicity. Ex vivo assays will determine which components of the stem cells are responsible for their immunomodulation, cytokine secretion or antigen-specific mechanisms. Using experimental autoimmune encephalomyelitis (EAE) as a mouse model of MS, we will:

Specific Aim 1. Determine the longevity of transplanted, immunomodulatory stem cells using 19-fluorine (19F) magnetic resonance imaging (MRI)

Specific Aim 2. Assess the ability of stem cells to protect glutamate-sensitive cells from excitotoxicity using stem cells using variable-delay multi-pulse chemical exchange saturation transfer (VDMP-CEST).

Specific Aim 3. Assess the ability of stem cells to modulate immunity using flow cytometry and immunostimulation assays of peripheral blood leukocytes.

The investigations proposed in this fellowship aim to develop a clinically relevant therapy to reduce the paralysis in MS. The approaches used are readily translatable. MRI is already used clinically, thus scanner protocols can be adapted for 19F MRI and VDMP-CEST. Mesenchymal stem cells are already undergoing clinical trials for immunomodulation. The source of glial progenitor cells used in this proposal is currently FDA-approved to be used in clinical trials. Furthermore, the strategies in these studies can be adapted to develop therapies for other neurological disorders.

Zhao Wei, Ph.D.
Johns Hopkins University
Mentor: Sharon Gerecht, Ph.D.
Award Amount: $130,000
Disease Target: Vascular Disease

3D Printing Vascularized Cardiac Constructs

The circulation of blood vessels in cardiac tissue is responsible for the blood supply to sustain heart functions. The complex cardiac tissue architecture depends on perfused vascular networks that provide necessary nutrient and waste transport within bulk myocardial tissues. Heart failure including ischemia and subsequently myocardial infarction occurs when the blood supply to heart is impeded by damaged vasculature. The capability to manufacture three-dimensional (3D) vascularized myocardial constructs that replicate the native vascularized cardiac tissue would enable multiple advanced applications in solving the current impact of cardiac dysfunctions. Such a system will provide platform for in vitro disease modeling and drug screening as well as construct for tissue repair and regeneration. The proposed research aims to develop a 3D myocardial construct with highly organized vessels in vitro by adopting the extrusion-based 3D bio-printing/molding technology. Following this strategy, we will precisely print the vasculature cell-laden inks into the supporting mold of myocardial construct by computer control, patterning the naturally branched and hierarchical vessels in cardiac tissues. Two pivotal components are required including cell source for printing and the selection of material as bio-inks and supporting mold. Human induced pluripotent stem cells (hiPSCs) possess the ability to differentiate into both early vascular cells (EVCs) and cardiomyocytes, which can be used as the stem cell source in building the 3D cardiovascular patches. Polymeric hydrogels have been broadly applied as 3D matrix, due to their biocompatibility and mechanical similarity to the native extracellular matrix (ECM) of many tissues. Importantly, hydrogel must possess both shear-thinning (printable) and self-healing (accommodating) properties to be used for 3D printing as bio-inks and supporting mold respectively. In this proposal, we seek to develop a novel bio-functional gelatin-based hydrogel with both shear-thinning and self-healing properties, cross-linked by oxidized dextran (Odex) through dynamic Schiff base reaction. We hypothesize that this novel hydrogel system can be used as suitable scaffold to separately encapsulate hiPSC-EVCs and cardiomyocytes, and then establish the 3D vascularized myocardial patches in vitro by directly printing EVCs-laden hydrogel inks into cardiomyocytes-laden hydrogel mold. We will culture the printed vascularized myocardial construct and characterize the cellular organization and function including the growth of vascular networks and the maturity and contractility of cardiomyocytes. The proposed research strategy involves a combination of biomaterials science and technique and stem cell biology for cardiac tissue regeneration. The impact of the study is the development of a 3D cardiac construct with highly organized vasculature in vitro that can mimic native tissues, and their potential utilization in treatment of heart failure by transplantation, building disease model or drug screening. We will also confirm promising potential of our novel approach for future clinical use of human stem cell-based vascular and cardiac tissue engineering. This engineered cardiovascular model will advance our knowledge about blood vessel assembly and cardiac functions during regeneration, help us translate the knowledge into clinical applications, and benefit healthcare in Maryland and our society.

Completed Grant Awards

A-Lien Lu-Chang, Ph.D.
University of Maryland, Baltimore
2014 Exploratory Award Budget: $230,000
Disease Target: Osteoarthritis and Other Diseases

The Effects of Histone Deacetylation and DNA Demethylation on Somatic Cell Reprogramming

Expression of defined sets of transcription factors can reprogram somatic cells to induced pluripotent stem cells (iPSCs). This technology holds enormous potential as iPSCs can be used as an alternative source for regenerative medicine. However, the low efficiency and the integration of viral transgenes in generating iPSCs impede the use of this method for biomedical applications. Because ectopic expression of OCT4 pluripotency regulator alone with small molecules can produce iPSCs, we focus on the stimulation of endogenous OCT4 gene expression using factors. Our ultimate goal is to produce iPSCs with only small molecules. Our results demonstrate that the efficiency of iPSC formation can be enhanced by reactivating OCT4 through altering its epigenetic state.

Histone modification and DNA methylation control epigenetic gene expression and are key factors in somatic cell reprogramming. SIRT1 is a member of the sirtuin family of NAD+-dependent histone/protein deacetylases (HDACs). SIRT1 deacetylates histones, many DNA repair enzymes, tumor suppressor p53, and other proteins. Interestingly, SIRT1 expression is higher in stem cells than in differentiated cells. It has been shown that SIRT1 can facilitate iPSC generation from mouse embryonic fibroblasts through deacetylating p53 and Sox2. Using a luciferase reporter system in human keratinocytes (NHEK), we show that sirtinol (a pan sirtuins inhibitor) inhibits OCT4 expression by 10-fold. SIRT1 overproduction can enhance OCT4-Luc expression by 3-folds. In addition, expression of OCT4-Luc is nearly abrogated in sirt1 knockout mouse embryonic fibroblast (MEF) cells. Similar results were observed with a GFP reporter system.

To further investigate the role of SIRT1 on gene expression of pluripotency factors and iPSC formation, we transfected NHEK cells with episomal Okita plasmids expressing reprogramming factors OCT4, KLF4, SOX2, and MYC (Keisuke Okita. et.al. Nature Method, 2011. 8: 409-412). SIRT1 overproduction enhances the transcription of all plasmid-derived reprogramming factors. However, SIRT1 only stimulates endogenous OCT4 expression, but not endogenous KLF4, SOX2, and MYC. Moreover, iPSCs are generated more efficiently when SIRT1 is overexpressed. The number of iPSC-like colonies increased by 7-fold from NHEK cells co-transfected with pECE-SIRT1 plasmid plus Okita plasmids as compared with that from cells co-transfected with cloning vector plus Okita plasmids. The iPSC-like colonies derived from pECE-SIRT1 plus Okita plasmids last longer than those derived from Okita plasmids alone. These colonies are bona fide iPSCs because they express the key stem cell markers TRA-1-60 and OCT4.

Our data indicate that SIRT1 enhances somatic cell reprogramming, in part, through enhancement of gene expression of endogenous OCT4 and ectopic transcription factors. Future development using SIRT1 activators and small molecules may eliminate the requirement of any transcription factors for iPSC production. Our novel approaches will not only increase the efficiency of iPSC induction but also generate iPSCs with high genomic stability. The genomic stability of iPSCs should be stringently maintained for the safety in tissue replacement applications. SIRT1 plays a pivotal role in controlling genomic stability by deacetylating DNA repair enzymes and tumor suppressor p53. By overproducing SIRT1 or using SIRT1 activators, the derived iPSCs should be highly proliferative, tolerant to oxidative stress and DNA damage, and highly similar to embryonic stem cells. Our finding will take steps closer to generate high quality iPSCs for regenerative medicine.

Jonathan Dinman, Ph.D.
University of Maryland, College Park
2014 Exploratory Award Budget: $230,000
Disease Target: Anemias, Hematopoietic Stem Cell Transplantation, Transfusion

Directed Delivery of Therapeutic RNAs into Hematopoietic Stem-Progenitor Cells

The purpose of this project was to develop an SDF1-RBD based ChemoARP that will enable researchers and clinicians to specifically deliver therapeutic RNAs into HSPCs and hematopoietic cell lines. Specifically, we designed a clone that fused the protein coding sequence of the SDF1 chemokine with the RNA binding domain (RBD) derived from the hepatitis B virus (HBV). The clone was produced and confirmed by DNA sequence analysis. It was then linearized and transfected into the yeast Pichia pastoris in such a way that it became stably integrated into the P. pastoris genome. This was confirmed by genetic and polymerase chain reaction (PCR) analysis of the P. pastoris chromosomal DNA. Next, we induced production of the recombinant SDF-RBD protein by addition of 1% methanol to cell cultures. This caused production of the protein, which was excreted from the cells into the surrounding medium. The recombinant protein was then purified from the medium using a simple 1 step anion exchange chromatography method. Purified protein was analyzed by using SDS polyacrylamide gel electrophoresis (SDS-PAGE) This is shown in Figure 1. The ability of the purified recombinant protein to function as an RNA transfection reagent was assessed by monitoring the ability of this reagent to knock down expression of a target gene in Jurkat cells that express the receptor for SDF1 (the receptor is called CXCR4). Figure 2 shows that performed as well as or better than the commercially available HiPerfect transfection reagent. From that point, we delivered the reagent to the laboratory of our collaborator, Dr. Curt Civin (UMB). They are currently characterizing the ability of this reagent to deliver miR-144 and miR-451 into haematopoetic stem precursor cells to regulate human erythropoiesis by targeting the RAB14 mRNA.
The crucial take home from this report is as follows: We successfully developed a strain of P. pastoris that produces the recombinant SDF1-RBD reagent that is capable of delivering small RNAs into cells that express the CXCR4 chemokine receptor. Having established this technology, it is now being characterized for its therapeutic applications.

Miroslaw Janowski, M.A., M.D., Ph.D.
Johns Hopkins University
2014 Exploratory Award Budget: $226,259
Disease Target: Leukodystrophy, Demyelination in the CNS

Magnetic Resonance Imaging of Myelination by Transplanted Glial Restricted Precursor Cells

Initially, we have established an in vivo multi-contrast MRI-based method to evaluate myelination produced by transplanted hGRP cells in the mouse brain. To achieve this goal, we have performed longitudinal studies of immunodeficient shiverer (shi/shi) x rag2-/- mice with transplanted hGRP cells using an array of MRI techniques and at several time points. This is important because non-invasive imaging tool is valuable for monitoring myelin repair by transplanted cells. We have evaluated the performance of conventional T2-weighted MRI (T2-w), magnetization transfer (MT) imaging, diffusion tensor imaging (DTI), diffusion kurtosis imaging (DK), and quantitative MT, and the ages of the transplanted animals ranged from 100 days post-transplantation to 600 days post-transplantation. Our results suggest that T2-w and MT imaging are most sensitive to detect myelination by transplanted hGRP cells. In comparison, DTI and DKI, although shown by numerous studies to be sensitive to the integrity of myelin, failed to detect myelination by the hGRPs in our model. Furthermore, we found that T2-w and MT imaging can detect myelination at 400 days post-transplantation, whereas histology showed robust myelination by the hGRPs as early as 200 days. Since, the regular DTI and DKI surprisingly failed to provide convincing results, we have applied recent advances in diffusion MRI to dig deeper into microstructure of changes associated with myelination from hGRPs in the corpus callosum of transplanted mice. The relationship between the non-invasive imaging findings and the underlying microstructural changes may provide additional insights for deciphering end improving therapeutic strategies and may be useful for guiding future clinical trials. Quantitative analysis of FA in the corpus callosum revealed slight differences between experimental groups with the highest anisotropy values in WT, the lowest values in shiverer, and intermediate values for transplanted mice. ADC, axial and radial diffusivities increased significantly in transplanted mice compared to WT and shiverer; while MK decreased in the shiverer and transplanted mice as compared to WT. The NODDI parameters revealed additional information separating the axonal and other cellular compartments compared to conventional diffusion tensor imaging. The cellular (nonaxonal) volume fraction increased in the shiverer mice compared to WT, which was further elevated in the Tx mice at 80wks; the axonal volume fraction showed an opposite trend. Another NODDI parameter, the ODI, which relates to the degree of orientation dispersion of axons, increased in the CC of shiverer mice, and returned to normal in the transplanted shiverer mice. DAPI staining showed increased cell density in the shiverer mice compared to WT, and that density markedly reduced in the transplanted mice. However the staining intensity in DAPI remained higher in the transplanted mice compared to WT mice. Due to the larger cell size of transplanted compared to host cells, even if the cell density/number became comparable to the WT mice, the overall extra-axonal volume fraction could still remain elevated in the transplanted mice. While imaging of stem cell-based myelination in shiverer model has been the major purpose of the study, there were previously reported spontaneous myelin changes in some aged mice based on post-mortem studies. We have found significant variability of DTI parameters among scanned animals, which confirm our hypothesis and can serve as a preliminary data for future studies targeting aged populations.

Manoj Kumar, Ph.D.
Johns Hopkins University
Mentor: Ted Dawson, Ph.D.
2014 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Parkinson's Disease

Human Dopaminergic Neuronal Loss Due To Parkin Insufficiency: Relevance to Parkinson's Disease

Objective: Generation of isogenic human parkin PD pluripotent stem cell lines. Characterization of mitochondrial function, deficits and biogenesis in the human parkin PD pluripotent stem cell lines. To rescue mitochondrial defects in hDA neurons by generating PARKIN PARIS double KO lines. Progress Summary/Accomplishments: We have generated two Parkin KO hESCs through TALE nuclease technology. These Parkin KO hESCs were validated by southern blot, RT PCR and Western blot. We differentiated Parkin KO lines to DA neurons with high efficiency using the floor plate method. We found fewer dopamine neurons present in Parkin KO lines compared to WT isogenic control. Parkin KO DA neurons were analyzed for mitochondrial quality control. We used confocal microscopy to assess mitochondrial phenotype and found clustered mitochondria in DA neurons in Parkin KO lines. Live dopaminergic culture was accessed for mitophagy. Differentiated cells were infected with lentivirus encoding mitokiema that labels mitochondria that are undergoing mitophagy. Less mitophagy was recorded in Parkin KO lines. Differentiated dopaminergic cultures were accessed for mitochondrial function using the XF analyzer (Sea Horse assay) and we found lower respiration in the Parkin KO cultures. We found PARIS level elevated in Parkin KO lines, this protein is accumulated in PD patient and affects DA neurons degeneration. Dopamine neurons cultures were assessed for mitochondrial biogenesis by labeling with Cox8a gene using SNAP tag technology. We found less mitochondrial biogenesis in Parkin KO derived dopamine cultures. The experiment were verified by Puromycin SUnSET assay, a nonradioactive method to monitor protein synthesis. We have generated Parkin and PARIS double KO lines, which rescue DA neurons loss compared to Parkin KO lines. Future Activities: Our next aim is to investigate mitochondrial biogenesis in Parkin KO lines. This will be done on 60 days differentiated dopaminergic culture. Preliminary experiments are encouraging. The experiments are already going on. We have generated PARKIN PARIS double KO lines using CRISPR/Cas9 to rescue DA neurons. The double KO lines will be used for evaluating mitochondrial function and biogenesis in dopaminergic neurons.

Publications/Presentations: Oral Presentation – The 7th annual Maryland Stem Cell Research Symposium, December 2014 Silver Spring, MD; Poster Presentation - The 7th annual Maryland Stem Cell Research Symposium, December 2014 Silver Spring, MD; Poster Presentation - The 8th annual Maryland Stem Cell Research Symposium, May 2016, Bethesda, Washington, DC

Translation Potential of the project: This study will provide a genuine working model for PD as it will have human genetic background. It can provide in vitro platform for drug screening for PD and its pathway especially in early intervention of PD.

Nicholas Maragakis, M.D.
Johns Hopkins University
2014 Exploratory Award Amount: $230,000
Disease Target: Amyotrophic Lateral Sclerosis

IPSC-Derived Neurons from Amyotrophic Lateral Sclerosis Patients to Study Disease Progression

The use of human induced pluripotent stem cell (IPSC)-derived neural cells from individuals with motor neuron diseases has generated enthusiasm for their potential as tools to understand disease mechanisms. Their value may be particularly relevant for motor neuron disorders, like amyotrophic lateral sclerosis (ALS), where the etiology of the disease, in the majority of patients, is unknown and accessibility to spinal cord tissues for analysis is not possible. In this study, we utilized iPSC-derived motor neurons from 6 subjects with SOD! mutations (3 $SOD1^{A4V}$ and 3 $SOD1^{090A}$) and 4 control subjects. We also incorporated 9 iPSC-derived neuron lines from sporadic ALS patients. We were able to generate neurons of significant purity, with an enriched subpopulation of motor neurons which were stable in culture for over 100 days. When we targeted a variety of ALS relevant regulatory pathways, we were able induce cell death in a dose-dependent manner and observe differences in the vulnerability of SOD I ALS iPSC-derived neurons when compared to control iPSC-derived neurons. However, we were not able to detect statistical differences between the sporadic ALS groups, according to disease progression or site of disease onset. These data demonstrate our capacity to stably maintain numerous iPSC-derived neuronal lines in culture and use a biochemical assay of cell death to reliably analyze susceptibility to cellular stress in both familial and sporadic forms of ALS. We were able to validate this model's potential for serially assessing MN vulnerability to a variety of cellular insults and were able to show an ALS-specific vulnerability amongst these SOD1 ALS producing lines. However, we were not able to dissect, in this relatively large sampling, a similar response in iPSC-derived MNs from sporadic ALS patients. These observations raise the possibility that other factors may be contributing to MN death in vitro including non-neuronal contributions to cell death, vulnerabilities unique to ALS genotype (TDP43, C90RF72), or perhaps the different conditions and timepoints utilized in our study. Indeed, recent work suggesting that iPSC-MNs derived from ALS patients have more similarities to fetal spinal tissue rather than adult spinal motor neurons suggests that methods for maturing these cell types may help the fidelity of translational data for ALS therapeutics14 This study also highlights the potential limitations of sampling individuals with sporadic ALS and suggests that larger sample sizes and additional phenotypic and neuronal subtyping may offer the most promising opportunities for recapitulating disease in vitro. This work is currently in preparation and titled: „Pluripotent Stem Cell-Derived Motor Neurons from Mutation Specific Familial but not Sporadic ALS patients are Susceptible to Cellular Stress"

Ke Ren, M.D., Ph.D.
University of Maryland, Baltimore
2014 Investigator Award Budget: $690,000
Disease Target: Chronic Pain Therapy

Mesenchymal Stem Cells for Chronic Pain Therapy

Chronic pain affects millions of people and is a major health problem. It costs the US over $500-650 billion each year in health care and lost productivity. It is also recognized in the State of Maryland that chronic intractable pain is a debilitating condition and may often be inadequately treated. The current treatment for chronic pain conditions such as neuropathic and musculoskeletal pain is unsatisfactory and there is an urgent need for searching and developing alternative and effective pain therapy. Multipotent stromal cells, or mesenchymal stem cells, can be derived from a variety of sources such as bone marrow, adipose tissues, the dental pulp, the umbilical cord and muscle. Mesenchymal stem cells have shown encouraging results in treating neurological disorders in clinical studies. Interestingly, mesenchymal stem cells appear to have potential to treat chronic pain conditions. This project has been designed to study potential pain-relieving (antihyperalgesic) effect of bone marrow stromal cells (BMSCs), a major type of multipotent mesenchymal stem cell, in preclinical model of persistent pain. We hypothesized that BMSCs produce the pain-relieving effect through immune interactions and subsequent activation of endogenous opioid system.

Aim 1. To characterize the effects of BMSCs and test the hypothesis that BMSCs engage the endogenous opioids and regulate cytokine/neuronal responses to injury. We observed that human (h) BMSCs produced antihyperalgesia in rats. In rats receiving ligation injury of the L5 spinal nerve, primary hBMSCs were infused through a tail vein (1.5M cells/0.2 ml). After nerve injury, rats exhibited a reduction in paw withdrawal latency, a measure of nociception, to a painful thermal stimulus. Compared to rats receiving infusion of the medium, the paw withdrawal latency of hBMSC-treated rats was significantly increased after BMSC infusion during the 8-week test period, indicating antihyperalgesia. Consistently, mechanical pain hypersensitivity, assessed with an electronic von Frey unit, was also reduced in hBMSC-treated rats. Further, nerve injury-induced aversive behavior was reduced, as shown in the conditioned place-avoidance test. Our previous results suggest that the attenuation of hyperalgesia by BMSCs involves a contribution from endogenous opioids. We performed multiple biochemical analyses to examine the effect of BMSCs on opioid receptor expression in a major structure of the brainstem descending pain modulatory circuitry, the rostral ventromedial medulla (RVM). Rats received ligation of the tendon of the masseter muscle, a model of myogenic orofacial pain and BMSCs were infused at 7 d after injury. In rats receiving BMSCs, the levels of mu-opioid receptor (MOR) mRNA and proteins were significantly increased at 1 week and 8 week after the treatment, compared to rats receiving control injection. These results are consistent with behavioral observations and support the view that BMSCs activate the endogenous opioid function to produce its longlasting pain-relieving effect.

Aim 2. To test the hypothesis that upregulation of opioid receptors and attenuation of persistent pain by MSC is mediated by immune mediators related to BMSC infusion. Previous studies indicate that primary, but not 20 Passage-BMSCs, produced pain relief, suggesting altered BMSC genotype during culturing. We observed differential expression profiles of major cytokines/chemokines and receptors between the primary and 20 Passage-BMSCs. Among the 84 genes examined, 50 in primary BMSCs were expressed at a higher level in all 3 samples (≥2-fold) and 26 of them showed greater than 100-fold differences over the 20 Passage-cells. Six genes expressed lower (>2-fold) in primary BMSCs. A repertoire of genes for immune mediators and receptors exhibited higher levels of expression in primary BMSCs compared to 20Passage-BMSC, including genes for chemotaxis-inducing chemokines and their receptors, anti-inflammatory cytokines and receptors, proinflammatory cytokines and receptors, and components of the complement pathways. While higher expression of the anti-inflammatory cytokines and receptors is consistent with antihypergesia, genes of proinflammatory cytokines and complement components may contribute to the effect of BMSCs by promoting chemotactic interactions with immune cells. Differential gene expressions between antihyperalgesic primary and ineffective 20 Passage-BMSCs suggest a role of BMSC-derived chemokines in BMSC-produced antihyperalgesia. We evaluated a role of two key chemoattactants, CCL4 and CCL2-CCR2. RNAi of CCL4 and pharmacological antagonism of CCR2 in BMSCs significantly reduced effects of BMSCs on MOR expression and hyperalgesia. These results indicate that BMSC-derived chemokines contributed to their antihyperalgesic effect. Our results provide the cellular mechanisms of BMSCs-produced pain relief and prompt translating this approach into clinical settings. The BMSC manipulation may be an efficient way to engage the endogenous opioid system, which may provide a novel approach for the management of chronic pain. Thus, our findings contribute to the advancement of stem cell medicine and formulation of alternative and effective stem cellbased pain management.

Lipeng Tian, Ph.D.
Johns Hopkins University
Mentor: Yoon-Young Jang, M.D., Ph.D.
2014 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Multiple

Human Stem Cell based Model of Alcoholic Liver Disease for Regenerative Therapy

The goal of this study is to develop stem cell models for alcoholic liver disease and to investigate how alcohol influences normal liver development in human. We have made a siginificant progress for aim 1 and aim 2 for the two years of funding. Alcohol consumption has long been associated with a majority of liver diseases and has been found to influence both fetal and adult liver functions. In spite of being one of the major causes of morbidity and mortality in the world, currently, there are no effective strategies that can prevent or treat alcoholic liver disease (ALD), due to a lack of human-relevant research models. Recent success in generation of functionally active mature hepatocyte-like cells from human-induced pluripotent cells (iPSCs} enables us to better understand the effects of alcohol on liver functions. We have examined the effects of alcohol exposure on multistage hepatic cells derived from human iPSCs, in an attempt to recapitulate the early stages of liver tissue injury associated with ALD. We exposed different stages of iPSC-induced hepatic cells to ethanol at multiple pathophysiological concentrations. In addition to stage-specific molecular markers, we measured several key cellular parameters of hepatocyte injury, including apoptosis, proliferation, and lipid accumulation. While alcohol has little effect on endoderm development from iPSCs, it reduces formation of hepatic progenitor cells during early hepatic specification. The proliferative activities of early and mature hepatocyte-like cells are significantly decreased after alcohol exposure. Importantly, at a mature stage of hepatocyte-like cells, alcohol treatment increases two liver progenitor subsets, causes oxidative mitochondrial injury and results in liver disease phenotypes (i.e., steatosis and hepatocellular carcinoma associated markers} in a dose dependent manner. Some of the phenotypes were significantly improved by antioxidant treatment. These results suggest that fetal alcohol exposure may impair generation of hepatic progenitors at early stage of hepatic specification and decrease proliferation of fetal hepatocytes; meanwhile alcohol injury in post-natal or mature stage human liver may contribute to disease phenotypes (a part of the results have been published as indicated in publications below, and most recent results have been accepted for publication; title is „Alcohol increases liver progenitor populations and induces disease phenotypes in human iPSC-derived mature stage hepatic cells“). This human iPSC model of alcohol-induced liver injury can be highly valuable for investigating alcoholic injury in the fetus as well as understanding the pathogenesis and ultimately developing effective treatment for alcoholic liver disease in adults.

Stephen Wolpe, Ph.D.
Orgenesis, Inc.
2014 Pre-Clinical Award Budget: $406,431
Disease Target: Diabetes

Autologous Insulin Producing (AIP) Cells for Diabetes

We started the project by successful technology transfer (large-scale expansion of liver cells, transdifferentiation process and scale-up as well as PCR, ELISA and flow cytometry release assays) from the Ferber laboratory at Sheba Medical Center in Israel to Noble Life Sciences in order to reproducibly make and assay liver-derived autologous insulin producing (AIP) cells at the scale needed for in vivo animal work. We then performed original work in developing the nude rat streptozotocin diabetes model as well as developing techniques to infuse 20x10^6 AIP cells into the portal vein of the rat liver in a safe and reproducible manner. This involved multiple iterations, comparing dose responses of single and multiple dose streptozotocin (STZ) before settling on a single-dose model that gives consistent and long-lasting diabetes on the order of several weeks. We also successfully treated STZdiabetic nude rats with transdifferentiated human-liver derived AIP cells and were able to maintain them for weeks. Serum samples from these studies show secretion of C-peptide indicating successful insulin secretion into the bloodstream. We completed our goal of testing the transdifferentiation capacity of normal livers compared to cell obtained from the livers of Type 1 Diabetes (T1D) patients. Data shows that liver cells from T1D patients underwent successful transdifferentiation. Gene expression analysis for the ectopic and induced pancreatic genes was slightly lower in T1D cells than those of the healthy donors, with the exception on Glucagon expression, which was higher in T1D livers. We performed immunogenicity tests by mixed lymphocyte reaction through the co-culture of AIP cells with PBMC from the blood from the same donor. We found no increase in the number of immune cells indicating that AIP cells have low immunogenicity. Biodistribution studies used GMP-like AIP cells manufactured at PALL Life-Sciences near Boston. Several weeks after implantation, we looked for the presence of human cells in rat organs. For this purpose, the qPCR technique was chosen for its high sensitivity. Human Alu sequences, in the presence of Sybr Green as reporter dye, were selected for testing. Kidney samples showed low but positive and specific amplification of human sequences, suggesting a negligible number of AIP cells ended up in the kidney. With Maxcyte we have created the mRNA vectors and compared the timing of electroporations to the viral infections. Because mRNA is instantly translated whereas viral vectors take additional time before the mRNA appears finding the optimal timing was crucial for the Maxcyte process. The current plan is to establish operations in Maryland and to conduct the additional studies requested by CBER/OCTGT after the pre-IND meeting in anticipation of IND submission for US clinical trials. Overall the plan is to have 10 patients in phase 1, 40 patients in phase 2 and 200 patients in phase 3.

Zijun Zhang, M.D., Ph.D.
MedStar Health Research Institute
2014 Exploratory Award Budget: $223,779
Disease Target: Bone Defect

Enhancing the Incorporation of Bone Allograft with Circulating Mesenchymal Stem Cells (MSCs)

Bone allograft, the bone tissue harvested from a donor, is widely used in orthopaedic and reconstructive surgery but its clinical outcome is often jeopardized by complications, such as non-union, fracture and infection. For patients' safety and reducing graft immunogenicity, current tissue banking practice processes and sterilizes bone allograft vigorously but, at the same time, the graft loses viable cells. It is generally believed that a nonviable bone allograft is the source of complications. The regenerative capacity of mesenchymal stem cells (MSCs) offers an opportunity to revitalize bone allograft. The long-term goal of the current project is to improve the clinical outcome of bone allograft with application of MSCs. The objective of this project is to investigate a new strategy, which is based on the molecular biology of fracture healing, of delivering MSCs to bone allograft. Specifically, this study aims to 1) enable allograft to attract stem cells by incorporation of molecules that attract MSCs during fracture healing and 2) examine whether MSCs administrated intravenously enhance the healing of bone allograft, when it is incorporated with the molecules attracting MSCs, in mice. Among several selected molecules and various combinations, a combination of molecules stem cell derived factor- (SDF-1) and platelet derived growth factor BB (PDGF-BB) were the most effective in attracting MSCs in a tissue culture setup that evaluates the migration of MSCs. Accordingly, SDF-1 and PDGF-BB were dissolved in degradable gel and coated on bone allograft prepared from mouse femora. Human MSCs were also isolated from bone marrow. Surgery was performed on immunodeficiency mice to implant bone allograft coated with or without SDF-1 and PDGF-BB. The animals that received bone allograft were intravenously administrated with human MSCs or saline weekly, for three weeks. After 8 weeks, bone allograft healed with the host bone in all mice. The group of mice that were implanted allograft, which was coated with SDF-1 and PDGF-BB, and injected with MSCs had the largest bone volume, comparing with other study groups. · Impact on public health and translational potential of the project
This study demonstrated that, when bone allograft coated with SDF-1 and PDGF-BB, intravenous administration of MSCs enhanced new bone formation around the allograft and host bone. Clinical translation of this strategy of stem cell application is highly feasible and potentially reduces clinical complications of bone allograft.

Srinivasa Raju Datla, Ph.D.
University of Maryland, Baltimore
Mentor: Sunjay Kaushal, Ph.D.
2015 Post-Doctoral Fellowship Award Amount: $110,000
Disease Target: Heart Defect, Myocardial Infarction

Allogeneic Safety Testing of c-Kit+ Cardiac Stem Cells

Specific Aim 1: Address the Immunological profiling of human and rat c-kit+ CSCs and in vitro investigation of allogeneic reactivity. For this aim the phenotypic profiling of human and rat c-kit+ CSCs has performed. Rat c-kit+ CPCs (rc-kit+ CPCs) were isolated from male Wistar Kyoto (WKY) rat hearts and human c-kit+ CPCs (he-kit+ CPCs) were isolated from adult coronary artery bypass graft patient's right atrial appendage. In vitro, both he-kit+ CPCs and rc-kit+ CPCs expressed MHC class I but not class II or co-stimulatory molecules CDSO and CD86. In rat mixed lymphocyte reaction (MLR) assay, allogeneic and syngeneic rc-kit+ CPCs elicited minimal T-cell proliferation compared to xenogeneic hekit+CPCs. In human MLR assay allogeneic he-kit+ CPCs elicited minimal T-cell proliferation, which is similar to cardiosphere-derived stem cells (CDCs) and mesenchymal stem cells (MSCs). In addition to hypo-immunogenic potentials, c-kit+ CPCs have shown to decrease the T-cell proliferation in stimulated condition.

Specific Aim 2: Evaluate the rat and human c-kit+ CSCs immune response and cardiac functional recovery potentials. To further evaluate in vivo allogeneic, functional potentials for c-kit+ CPCs in the MI model, rc-kit+ CPCs derived from WKY rats were transplanted in WKY and Brown Norway (BN) female rats for the syngeneic and allogeneic groups, respectively. Similarly, for the xenogeneic group, he-kit+ CPCs were transplanted in BN male rats. Both syngeneic, and allogeneic rc-kit+ CPCs groups elicited minimal local, systemic and humoral inflammatory response compared to the xenogeneic c-kit+ CPCs group. In addition, the allogeneic and syngeneic rc-kit+ CPCs groups significantly recovered cardiac function (EF, SV, and CO) compared to the xenogeneic c-kit+ CPCs group, which had no functional recoverability. Histological analysis further revealed that allogeneic and syngeneic rc-kit+ CPCs groups had significantly decreased scar size by increasing cardiomyocyte proliferation, endogenous c-kit+ CPC recruitment and increased neoangiogenesis. Interestingly it is also noticed that there is an increase in FoxP3 and M2 macro phages in the allogeneic and syngeneic groups compared to xenogeneic group. These observations further confirmed in the in vitro conditions. This data suggests that c-kit+ CSCs might have an immunomodulatory property in addition to their direct role in cardiac structural improvements. Further investigation reviled that the c-kit+ CPCs could be decreasing the immune modulatory property via its secretory products i.e., exosomes and soluble factors. The project is concluded that the allogeneic c-kit+ CPCs are immune tolerant and produce minimal inflammatory response while maintaining their cardiac recovery potentials in immunologically divergent species. Observations from this project, for the fist time, report the minimal immunogenic response by c-kit+ CPCs and strongly support the development of an allogeneic c-kit+ CPC strategy for clinical trial applications. Overall in this project I successfully completed the proposed aims and extended the studies to evaluate the mechanism for immune-modulatory property observed.

Ted Dawson, M.D., Ph.D.
Johns Hopkins University
Mentor: Duojia Pan, Ph.D.
2015 Exploratory Award Budget: $218,500
Disease Target: Parkinson‘s Disease

Development of a Chimeric Human Mouse Model of Parkinson’s Disease

Specific Aim 1: Generation of human dopamine (DA) neurons in mouse via blastocyst complementation.
Specific Aim 2: Characterization of human (OA) neurons in the intact mouse brain
The Goals, Specific Aims and Impact on Biotechnology remained the same throughout the funding period.

Specific Aim 1:
We successfully established stable RUES1 and RUES2 cell lines expressing the fluorescent marker proteins, GFP or dsRED. These GFP or dsRED expressing ES cell lines were shown to have a normal karyotype and form embryonic bodies which contained three primary tissue layers. We imported and established mouse colonies of En 1^{+t-} and $Pitx3^{+t-}$ mice. We agenerated ground state human RUES cell lines, which were used for chimeric blastocyst complementation and stage-matching transplantation into gastrula-stage embryos from $En1_{-,-}$ or $Pitx3_{-,-}$ embryos. Several chimeric embryos were produced, the majority of which were heterzogotes for EN1 or Pitx3. A few homozygote chimeric embryos were also generated.

Specific Aim 2:
The chimeric human DA neurons were subjected to a variety and biochemical and electrophysiologic assays and were determined to be authentic midbrain DA neurons. A manuscript is being prepared describing these findings.

Translational Potential of Project: The research supported by this grant provides the resources to establish a chimeric human mouse model of Parkinson‘s disease. The success of this project has the potential to transform the study and treatment of PO by providing a new potentially robust and accurate model of PO pathogenesis in human PO that can be used for potential discovery of biochemical and/or molecular markers and new therapies for PD.

Peter Johnston, Ph.D.
Johns Hopkins University
2015 Exploratory Award Budget: $218,237
Disease Target: Peripheral Vascular Disease & Critical Limb Ischemia

Cell Impregnated Nanofiber Stent Sleeve for Peripheral Vascular Repair

Peripheral artery disease affects more than 8 million Americans. In the most severe form, critical limb ischemia (CLI), morbidity and mortality are high and treatment options are limited. Stem cell therapy may provide new options for treatment of CLI, but effects in clinical trials to date how mixed results. Many of the beneficial effects of stem cells are mediated by so called “paracrine factors” (PFs) that they release, which include growth factors, signaling molecules, and exosomes. To harness the beneficial effects of stem cell PFs to treat CLI we developed the “Cell-Impregnated Nanofiber Stent Sleeve” (CINSS), comprised of a nanofiber mesh applied to a clinical-grade vascular stent. The nanofibers incorporate and envelope stem cells, providing a protected environment from which cells may release their full spectrum of beneficial PFs to affected arteries over a sustained period. The major goals of our project were to develop and test the CINSS in a pre-clinical animal model of CLI. CINSS loaded with Mesenchymal Stem Cells (MSCs) may be crimped and expanded with only temporary effect on cell viability. In the first year of the project we showed: a) that crimping temporarily affects viability of cells contained in the CINSS, although does not do so permanently. To assess changes in viability following stent crimping, CINSS were then crimped and re-expanded. These results indicate that while crimping of CINSS results in an initial decrease in viability of contained MSCs, this effect is temporary and does not preclude the use of crimping in as required for intravascular CINSS delivery. CINSS successful implanted in live animal model. Using the same crimping technique, CINSS were applied to clinical angioplasty balloons and delivered to the superficial femoral artery (SFA) of adult rabbits using standard clinical technique. Contrast angiography after CINSS deployment revealed patent SFA and peripheral arteries with no evidence of dissection and preserved antegrade blood flow. These results demonstrated that the CINSS may be delivered to the rabbit peripheral vasculature as required for testing in our pre-clinical model. CINSS deployment in CLI model results in vascular thrombosis and occlusion. After showing CINSS delivery and deployment was feasible in the rabbit peripheral vasculature, we proceeded to test MSC-loaded CINSS in a model of CLI in which vascular coils are deployed distal to the stent to obstruct blood flow. While this technique was used previously with directly injected stem cells, with deployment of the CINSS following vascular coils the stented segment rapidly developed thrombotic occlusion. This was despite the use of anticoagulantion and dual-anti-platelet therapy as is standard in clinical practice. This issue persisted despite modification of the rabbit model. We believe this issue may be overcome by using a different animal model with larger peripheral vasculature and greater distal runoff. We are therefore exploring testing of the CINSS in a CLI model in the pig. Impact on Public Health / Translational Potential of Project: Peripheral artery disease and CLI are serious public health problems in need of novel therapies. We remain confident that with modifications to the CINSS platform and testing in a different animal model the approach of loading vascular stents with stem cells to promote release of beneficial PFs can significantly improve outcomes for patients with peripheral artery disease and CLI.

Yunqing Li, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2015 Exploratory Award Budget: $218,500
Disease Target: Spinal cord injury; Cell Replacement Therapy; Lineage Differentiation; MicroRNAs

Regulation of Oligodendrocyte Identity by MicroRNA Networks

Oligodendrocyte precursor cells (OPCs) as the predominant source of myelinating oligodendrocytes in the CNS have shown promise as a cellular therapeutic in animal demyelination models. However, primary OPCs are not readily available. Several approaches have been established to generate induced OPCs (iOPCs) from the fetal brain or human embryonic stem cells (hESCs) as well as from human induced pluripotent cells (hiPSCs). These approaches are time-consuming (75-180 days), and inefficient (20-75%), generating heterogeneous cells (oligodendrocyte and astrocyte) and tumorigenic potential (undifferented pluripotent cells), and thus have limitations for regenerative medicine. The goal of this project will optimize differentiation strategies for generating sufficient, homogenous, and safe autologous OPCs in a clinically applicable timeframe that can be used for cell replacement therapy for treatment of spinal cord injury and other CNS disorders. In this study, we have established an efficient hiPSCs-derived iOPCs protocol by combining transcription factor mRNA cocktail with the oligodendrocyte specification signals. We have demonstrated that the induction of transcription factors (Sox1 0, NKX2.2, olig2 and ASCL 1) in human iPSCs is sufficient to rapidly generate 04+ OL with an efficiency of up to 80-90% in 20-40 days. Most of these cells (90%) exhibited typical small bi-polar or tri-polar OPC-like morphologies, maintained their self-renewal capacity and expressed OPC-specific markers NG2, PDGFRa, Sox1 0 and 04. Moreover, these iOPCs maintained high proliferation as shown by 60% Ki67+ positive. These iOPCs can be stably expanded for more than 20 passages. Furthermore, we demonstrated that this protocol with some modifications can also be applied to generate iOPCs from human fibroblasts. Our protocol used messenger RNA delivery of transcription factor while circumventing issues related to insertion into the host genome, immune and tumor formation concerns previously associated with using lentiviral constructs in generation of patient-specific OPCs. Therefore, our protocol offers a safe and effective means of generating patient-specific iOPCs. These iOPCs are suitable for cell replacement therapy for treatment of dysmyelination or demyelination disorders such as spinal cord injury, multiple sclerosis and inherited leukodystrophies(X-ALD). This technology has been disclosed to John Hopkins Technology Ventures in preparation for IP protection and patenting (on July 11, 2016, #C14278).

Ileana Lorenzini, Ph.D.
Johns Hopkins University
Mentor: Rita Sattler, Ph.D.
2015 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Amyotrophic Lateral Sclerosis, Dementia

Role of Structural and Functional Changes of Dendritic Spines in Patient-Derived C9ORF72 iPS Neurons

The discovery of the hexanucleotide repeat expansion GGGGCC (G4C2) in the C9orf72 gene represents up to 10% of sporadic and up to 40% of the familial ALS cases. In vitro and in vivo disease models revealed several disease mechanisms: protein loss of function, toxic RNA gain of function, generation of dipeptide repeat proteins via nonATG initiated (RAN) translation, and impairment of the nuclear-cytoplasmic transport. Studies from different laboratories have identified RNA interacting proteins that are sequestered to the G4C2 repeat expansion, including proteins involved in synaptic neurotransmission. In addition, human C9 patient-derived induced pluripotent stem cells differentiated into neurons (iPSNs) show changes in neuronal excitability and increased susceptibility to cellular stressors. Based on these pathogenic phenotypes we hypothesize that synaptic deficits are a consequence of the sequestration of critical proteins involved in synapse formation and function to the G4C2 repeat expansion. First, we performed morphological analysis of the iPSNs to confirmed alterations in dendritic morphology. C90RF72 iPS motor neurons show decreased spine density and altered spine morphology at different ages. The spine density in C90RF72 iPS motor neurons was reduced at both young Day 40 and old Day 55-60 differentiation time points. As the cells age, the proportion of mushroom spines increases in control cells so that mushroom spines become the most prevalent type of spine. As C90RF72 iPS cells age, stubby spines become most prevalent while the proportion of mushrooms spines does not change suggesting that cells are immature. In addition, C90RF72 iPS neurons show altered dendritic branching. We performed shall analysis profile in IPSNs and show an increase in average number of motor neurons dendrite intersections towards the radial distance from the center of the neuronal cell body. We also found a reduced expression pattern of synaptic protein synapsin-1 in C90RF72 young and old iPS motor neurons. In C90RF72 iPS cortical neurons, synapsin-1 and vglutl expression was also reduced. C90RF72 iPS neurons show signs of synaptic dysregulation as seen by decreased spine density and structural changes in dendritic branching (motor and cortical neurons) as well as for the altered expression pattern of synapsin-1. Our data suggest that synaptic dysfunction plays a role in C90RF72 pathogenesis and may explain the observed changes in neuronal excitability and increased susceptibility to stressors, which are likely to lead to cognitive impairment and neuronal cell death, as observed in C90RF72 ALS patients.

Hideki Uosaki, Ph.D.
Johns Hopkins University
Mentor: Chulan Kwon, M.S., Ph.D.
2015 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Heart

MicroRNA-based Maturation of Cardiomyocytes Derived from Human Pluripotent Stem Cells

We have investigated to understand molecular mechanisms by which cardiomyocyte maturation is regulated and to develop a new method to obtain mature cardiomyocytes from pluripotent stem cells in a lab, which is currently impossible to achieve. First, we studied the course how cardiomyocytes mature in a mouse heart using publically available database. In this study, we successfully demonstrated how dynamically gene expression changes, and possible causes of its regulation. Regulator genes , called transcriptional factors and target genes forms a sort of networks. Second, we developed a new method to tell how much maturation cardiomyocytes achieved, based on the gene networks. Third, using the new method, we found that pluripotent stem cell-derived cardiomyocytes matured to late-embronic to neonatal stages. These results were reported to highly regarded lifescience journal Cell Reports (Uosaki et al. Cell Rep, 2015). We further analyzed the data and compared to human data to see how similar mouse and human cardiomyocytes are in terms of gene expression. We demonstrated global similarity between the two. The results were reported to Genomics, Proteomics and Bioinformatics (Uosaki and Taguchi, GPB, 2016). The original idea of this project was to test if some candidate microRNAs, which are short RNA and do not code protein but inhibit other protein-coding messenger RNA, could enhance or inhibit cardiomyocytes maturation. The carndidate microRNAs were determined through the informatics study of cardiomyocyte maturation. As one of the candidate microRNA was repoted to involve in cardiomyocyte maturation by other group, we have planed to identify more candidate microRNAs by experimentally. Although the experiments were on the way, the PI was promoted to a new job outside of the US and the project was terminated, therefore it remains elusive if there are microRNAs that enhance or inhibit cardiomyocyte maturation.

Jiou Wang, Ph.D.
Johns Hopkins University
2015 Exploratory Award Budget: $218,500
Disease Target: Amyotrophic Lateral Sclerosis, Frontotemporal Dementia, and Alzheimer's diseases

Pathogenic Mechanisms and Intervention Targets in Neurodegenerative Disease ALS/FTD

Amyotrophic lateral sclerosis (ALS) is characterized by the loss of motor neurons and the most common mutation which causes ALS is the hexanucleotide repeat expansion (HRE) of C9orf72. It has been proposed that there are three possible pathogenic mechanisms; toxicity caused by dipeptide proteins or RNA from C9orf72 HRE and the haploinsufficiency of C9orf72. However, the current animal models for ALS do not fully represent the human disease. Therefore, establishment of suitable model systems for ALS can accelerate our study to achieve our goals. We are grateful for the support from an exploratory grant of MSCRF for the last two years, which will end in May of 2017. This starter grant has allowed us to build new expertise in apply stem cell technology in our laboratory's research as well as to train new stem cell researchers and technicians, which has contributed to the growth of stem cell-related research in Maryland. The work in the original proposal was designed to basic pathogenic mechanisms underlying expansion of the hexanucleotide repeat in C9orf72, a genetic abnormity that has recently been found to cause the most common form of ALS/FTD. Our work and others' have suggested that there are three possible pathogenic mechanisms: toxicity caused by RNA from C9orf72 HRE or dipeptide proteins and the haploinsufficiency of C9orf72. However, the current animal models for ALS/FTD do not fully represent the human disease. Therefore, establishment of suitable model systems for ALS/FTD can accelerate the studies needed to achieve our goals. We have utilized the ALS/FTD patient derived induced pluripotent stem cells (iPSCs) to study the toxicity of C9orf72 HRE. We have made important progress in studying the basic structural features of the C9orf72 nucleotide repeat and their interactions with disease-relevant protein targets in iPSC derived models. For example, together with our colleagues, we have discovered that G-quadruplex structures of the C9orf72 sense HRE RNA is a specific binding partner of the RanGAP protein, which plays in important function in nuclear transport. Several labs including ours have observed defects in nuclear transport in patient iPSC derived neuronal cells. Furthermore, we observed that a G-quadruplex-modulating drug could potentially modulate C9orf72 HRE toxicity. TMTyP4, a porphyrin compound recognizing G-quadruplexes, has been shown to distort the HRE RNA G-quadruplex structures. We found that TMTyP4 could block the biochemical interaction between the repeat RNA, (G4C2)10, and the nuclear pore protein RanGAP1. Moreover, the drug ameliorated the nucleocytoplasmic transport defects (Zhang et al., Nature 2015). Our results demonstrate the power of combining biochemistry and molecular biology with in vivo experimental methods. Using the patient iPSC derived neuronal models, we are determining additional cellular sites injured by the toxic mechanisms of C9orf72 HRE. One of the mechanisms involved is the haploinsufficiency of C9orf72, since the HRE disrupts the expression of the C9orf72 protein. We discovered the C9orf72 protein is a central regulator of autophagy by characterizing its functions in knockout mice and mouse embryonic stem cells . We are also extending our results from mouse stem cells to human iPSC models. Furthermore, we are making additional important progress towards uncovering other pathogenic mechanisms and identifying promising therapeutic agents for C9orf72-linked diseases as well as preparing more manuscripts to publish these findings accordingly.

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2015 Exploratory Award Budget: $218,500
Disease Target: Parkinson’s Disease

Highly Efficient Conversion of Human iPS Cells to Dopaminergic Neurons by Synthetic Modified mRNAs

We proposed to develop the first synthetic-mRNA-induced strategy for highly efficient generation of midbrain dopaminergic (mDA) neurons from human induced pluripotent stem cells (iPSCs).

In **Aim 1**, we proposed to use synthetic mRNAs coding modified Atoh1 and other transcription factors for highly efficient mDA neuron differentiation, and characterize the functions of these neurons. We have developed a 5-day mDA neuron differentiation protocol using mRNA coding Atoh1 with defined phosphosite modification (referred to as Atoh1-SA). Compared to unmodified Atoh1, Atoh1-SA mRNA more efficiently induced mDA neuron generation from iPSCs. We have characterized the electrophysiological activity and dopamine release of these neurons. We have also established highly efficient drug screening platform using these mDA neurons. We are currently studying the function of these mDA neurons in treating the rat model for Parkinson‘s disease. More importantly, we have identified a novel regulator of Atoh1‘s activity in driving neuron conversion, which will be used to further improve our current mDA neuron differentiation method. We found that the non-muscle Myosin II (NM-II) protein complex binds to Atoh1 and promotes the activity of Atoh1. A manuscript as listed below will be submitted in two months. A patent covering this mDA neuron differentiation method using synthetic Atoh1 mRNA has been filed and licensed to Molecular Transfer, Inc. (MTI).

In **Aim 2**, we proposed to globally identify Atoh1‘s gene targets during mDA neuron conversion. We have performed ChIP-sequencing analysis to globally identify Atoh1-activated gene targets during iPSC conversion to mDA neurons. We found that Atoh1 directly transactivates mDA neuron identity genes. This result reveals how Atoh1 induces mDA neuron differentiation. Now, we are studying genes differentially regulated by modified and unmodified Atoh1 in order to further understand how phosphosite modification regulates the function of Atoh1. We are also studying how the NM-II complex modulates the function of Atoh1 in regulating mDA neuron identity genes. We found that inhibiting MYL9 and MYL10, two key components of the NM-II complex, suppresses the function of Atoh1 in activating TH and FoxA2, two mDA neuron identity genes. We are now testing if novel chemical compounds that activate MYL9 or MYL10 expression promote Atoh1-induced mDA neuron conversion. Positive results will likely provide novel strategies to optimize Atoh1-induced mDA neuron differentiation. We anticipate that these discoveries will lead to new publications and intellectual properties.

The cell differentiation strategy developed from this project significantly improves the production of transplantable mDA neurons from human iPSCs. This invention will facilitate the application of mDA neurons in disease modeling, drug testing and cell replacement therapy for Parkinson’s disease and other neurological disorders. The patent has been licensed to MTI, a biotech company in Maryland. MTI is currently marketing human iPSC-derived mDA neurons with ~30% purity (the GS-DOPA Kit, Cat. No.: GSK-4401). Our novel synthetic-mRNA-driven method rapidly generates iPSC-derived mDA neurons with >80% purity, significantly higher than the purity of mDA neurons currently offered by MTI. Thus, our patented technique will allow MTI to provide a highly competitive product that will likely boost their sales of mDA neurons and related culture media. Moreover, we are currently developing synthetic-mRNA-driven methods for generating other hard-to-achieve neural cells (e.g. motor neurons and oligodendrocytes) from iPSCs. This project has been funded by the 2017 MSCRF Discovery Grant. This on-going research will likely provide patentable inventions and licensing opportunities to MTI and other biotech companies, and in turn expand their product lines.

Sooyeon Yoo, Ph.D.
Johns Hopkins University
Mentor: Seth Blackshaw, Ph.D.
2015 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Obesity, Eating Disorders

Adult Hypothalamic Neurogenesis and Regulation of Feeding and Metabolism

Obesity is a severe public health problem in the developed world. Despite substantial progress in understanding hypothalamic neural circuitry regulating energy balance, much less is known about what is the source of the adult newborn neurons and how changes in the function and connectivity of these cells leads to obesity. Our group has identified median eminence (ME), which is mainly composed a specialized class of radial glial cells known as tanycytes, as a source of active neurogenesis in juvenile mice (Lee at al. 2012). Unlike other regions of the hypothalamus, the ME lies outside the blood brain barrier (BBB), allowing rapid and sensitive response to dietary-regulated blood-borne signals. In addition, tanycytes in the ME extend their processes to directly contact the permeable fenestrated capillaries. Given these facts, we hypothesized that tanycyte-derived neurogenesis plays a central role in control of body weight. This project was aimed at better understanding the mechanism by which tanycyte-derived adult neurogenesis regulates body weight, using the powerful tanycyte-specific inducible Cre recombinase line that our group previously generated (Pak at al. 2013), and also to determine whether these findings are directly applicable to humans using xenografting of human embryonic stem cell (hESC)-derived tanycytes. To determine the molecular identity of neurons in the ME, in the first year of the MSCRF fellowship, we optimized protocols for fluorescence in situ hybridization combined with immunohistochemistry, and this allowed us to determine the identity of the tanycyte-derived parenchymal neuronal subtypes. This proved to be a more practical and direct way of investigating the first question in our proposal than the use of the individual eGFP transgenic mice under the control of regulatory elements of subtype-specific marker gene as originally proposed. We found that we could selectively identify tanycyte-derived neurons using the Rax-CreERT2:Ai9 mice within normal chow or high-fat diet and screened molecular markers of major hypothalamic neuronal subtypes known to regulate feeding and body weight. We observed that tanycyte-derived TdTomato-positive cells expressed a number of these markers.

To confirm that these neurons are newly generated adult born neurons, we are currently measuring the BrdU incorporation into these newborn neurons using intraventricular osmotic pump implantation. We found that the levels of TdTomato expression in the labeled neurons were insufficiently strong to visualize their synaptic connections. In future studies, we plan to generate the RaxCreERT2;Ai34D mice, which express reporter gene fused to Synaptophysin in a Credependent manner, as we suggested in our research proposal. Next, we examined whether tamoxifen-mediated ablation of tanycyte-derived neurons modulate HFD-induced weight gain using Rax-CreERT2:Eno2-lox-stop-lox-DTA mice. We are currently collecting data to determine the physiological consequences of disrupting tanycytederived neurogenesis by analyzing total body content and indirect calorimetry. Moreover, we unexpectedly found that Eno2 is expressed not only mature neurons but also beta-subtypes of tanycytes, so that Cre-dependent induction of DTA expression led to not only a disruption of tancyyte-derived neurogenesis, but also a transient loss of beta tanycytes. We are now using these mice to distinguish the acute physiological effects resulting from the loss of beta tanycytes, in addition to the long-term effects of disrupting tanycyte-derived neurogenesis. During the second year of the MSCRF fellowship, we have begun conducting RNA-Seq analysis of tanycyte-derived cells in order to obtain a comprehensive profile of genes expressed in these cells, and to also identify changes in gene expression that occur in response to different diets in different sex and ages. This information is being used to identify genes that control neurogenic competence in tanycytes, and is being used to help guide and inform protocols for the directed differentiation of tanycytes from human ES cells. In the near future, this work enables to directly address the relevance of tancytye-derived neurogenesis to human disease, eventually pointing the way toward a novel therapeutic approach for treating obesity.

Ki-Jun Yun, Ph.D.
Johns Hopkins University
Mentor: Hongjun Song, Ph.D.
2015 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Schizophrenia, Autism & Mental Disorders

Modeling Neurodevelopmental Defects in Psychiatric Disorders Using iPSC-Derived 3D Cerebral Organoid

Establishment of cerebral organoid differentiation protocol: We have developed an improved protocol for generating the cerebral organoids with defined factors. The variations among different culture conditions are biggest obstacles to control the experiment. To resolve this, we successfully labeled control iPSC lines with GFP and experimental iPSC lines (15q11.2-del/dup) with RFP by lentivirus and perform mixed differentiation in the same cerebral organoid. Immunohistochemistry with different markers revealed that neural tube structures in cerebral organoids maintained typical features of developing cortex. We have generated multiple cerebral organoids with different doses of 15q11.2 with or without lentiviral labeling.

Characterization of neural progenitors in cerebral organoids with 15q11.2-del/dup: To examine dose-dependent effects of 15q11.2 on adherence junctions and polarity of neuroepithelial cells, we generated early-stage cerebral organoids (day 25–50) from 15q11.2-del/dup and control iPSC lines. Immunohistochemistry analysis showed that neuroepithelial tissues generated from control iPSCs using our cerebral organoid protocol exhibit structures reminiscent of the developing brain, with apical placement of Pax6+/Sox2+ neural progenitors and localization of aPKCl at the apical surface.

Examination of cortical layer formation in cerebral organoids with 15q11.2 CNVs: We analyzed the cellular localization of CTIP2+ deep layer neurons in mid-stage organoids (day 40-50) to examine initial migration potential of newborn neurons. The distance from the ventricle to each CTIP2+ soma was quantified among organoids from 15q11.2-del, 15q11.2-dup and control iPSC lines.. To do 'birth-dating' of newborn neurons for more accurate analysis of neuronal migration, we applied a pulse of thymidine analogue, EdU, into culture media 7 days before fixation and quantify migration distance of EdU+CTIP2+ neurons. Those results showed we can label different corical layer neurons separately with defined protocols.

Morphological analysis of migrating neurons: Migrating newborn neurons have typical polarized morphologies and subcellular organelles (Golgi apparatus, centrosome). To visualize the morphology of individual migrating neurons, we used GFP-expressing lentivirus or electroporation of GFP
construct to cerebral organoids. GFP+ neurons were analyzed by confocal microscopy and categorized based on their morphology (multipolar/unipolar/bipolar), and immunostained to examine polarization of centrosome (g-Tubulin, CEP15) and Golgi (GM130). We will classify the behavior of newborn migrating neurons in our forebrain organoid system.

Translational potential: This project is to establish a model system using cerebral organoids to pinpoint functional roles of genes within CNVs implicated in neuropsychiatric diseases. This strategy can be extended for investigation of other risk CNVs, helping other translational researches. In addition, this study on 15q11.2-del/dup in 3D culture system may aid in the establishment of robust phenotypic human cell-based assays and for future high-throughput screening of pharmacological drugs for neuropsychiatric disorders.

Allison Bond, Ph.D.
Johns Hopkins University
Mentor: Guo-Li Ming, M.D., Ph.D.
2016 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Schizophrenia, Depressive Disorders

Evaluating the Impact of Genetic Risk Factors for Psychiatric Disorders on Interneurons

Aim 1: **Compare the transcriptomes of GABAergic interneuron subtypes derived from human iPSCs.** We have optimized our GABAergic interneuron differentiation protocol, such that we get multiple subtypes of interneuron. I have begun doing single-cell RNA library preparation on these human interneurons, and recently began sending the libraries for sequencing.There are no changes in the approach. There are no anticipated problems or delays.

Aim 2: **To determine whether a mutation in DISC1 differentially affects the transcriptomic signature of human GABAergic interneuron subtypes.** We have differentiated DISC1 patient iPSCs using our GABAergic interneuron differentiation protocol and have not observed any gross differences compared to control patient iPSCs. I have begun doing single-cell RNA library preparation on these human interneurons, and recently began sending the libraries for sequencing. Preliminary results from functional cellular assays suggest that DISC1 patient iPSC-derived GABAergic interneurons have synaptic deficits similar to DISC1 patient iPSC-derived glutamatergic neurons. In the near future I will begin to compare transcriptomic signatures of synaptic function, as well. There are no changes in the approach. There are no anticipated problems or delays.

Dhruv Vig, Ph.D.
Johns Hopkins University
Mentor: Sharon Gerecht, Ph.D. & Sean Sun, Ph.D.
2016 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Vascular Diseases

Geometric Cues in the Establishment and Maintenance of Heterogeneous Stem Cell Colonies

We sought to investigate the effect of tension on the differentiation of pluripotent stem cells. We were able to successfully induce tension by confining stem cells on surfaces of various geometries, such as circles, squares, triangles, five-pointed and ten-pointed stars. We found that regions of high tension, for example the points of a triangle led to a higher differentiation rate then regions of low tension. These experimental results were then used in a machine learning algorithm to make predictions on the biochemical and mechanical parameters that control pluripotent stem cell differentiation. Our preliminary findings suggest that tension and density are two parameters that regulate a stem cell's fate decision.

Kathryn Wagner, M.D., Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2016 Exploratory Award Budget: $111,875
Disease Target: Muscular Dystrophy, Myopathy

A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation (Continuation)

Stem cell therapy is considered by many to hold the promise of a cure for chronic muscle disorders such as the muscular dystrophies. However, when stem cells are transplanted into dystrophic muscle, they do not typically survive nor form new muscle. Our hypothesis is that stem cells must begin engraftment in a more hospitable environment. We have developed an injectable biosynthetic scaffold for muscle progenitor cells derived from human induced pluripotent stem cells.

Our proposal had the following Aims:

Aim 1: Optimize engraftment potential of donor stem cells in a mouse model of muscular dystrophy (mdx mouse muscle)

Aim 2: Determine whether donor stem cells improve histopathology of mdx host muscle

Aim 3: Determine whether donor stem cells improve muscle function in mdx.

We created a multifunctional injectable biological scaffold composed of hyaluronic acid and decellularized skeletal muscle extracellular matrix. This material forms a cytocompatible hydrogel at physiological temperatures in vitro. When injected subfascially into both wild-type and dystrophic mdx mice, the hydrogel spreads across the entire muscle before complete degradation at three weeks in vivo. Transplantation of human myoblasts with the hydrogel promoted their survival in vivo, and delivery of myostatin inhibitor with the hydrogel increased its bioactivity in vivo.

Interestingly, the hydrogel modulates the local immune response. The hydrogel alone is associated with CD206+ macrophage polarization (macrophages that are associated with repair and regeneration) and elevated anti-inflammatory cytokine expression in both wild-type and dystrophic mdx mice. Co-injection of both hydrogel and myostatin inhibitor yields a significant increase in Foxp3+ regulatory T cells (cells that suppress immune responses in other cells) in the scaffold immune microenvironment – an effect not observed with any single component alone.

These results are encouraging for the development of a hospitable environment for cell transplantation for muscle disorders. However, there remains considerable work to be done as the yield of stem cell engraftment is still extremely low (less than 0.01%).