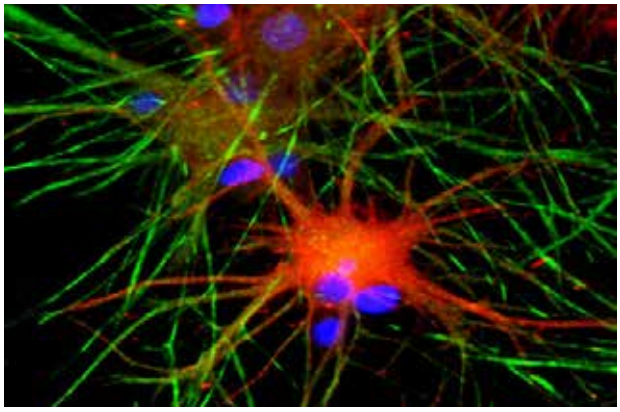
MSCRF

Maryland

STEM CELL RESEARCH FUND



Annual Report

Calendar Year
2015

Promoting State-Funded Stem Cell Research & Cures

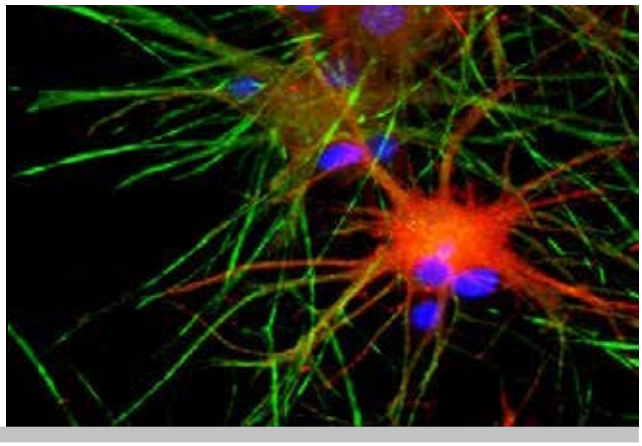


TABLE OF CONTENTS

MSCRF
Maryland Stem Cell Research Fund

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

David Mosser, Ph.D. – Vice Chair
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Department of Cell Biology and Molecular
Genetics, University of Maryland, College Park.

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

Rev. Kevin Fitzgerald, Ph.D.
(Appointed by the Governor)
Associate Professor, Department of Oncology,
Georgetown University Medical Center.

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.

Marye D. Kellermann, RN
(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.

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Assistant Professor, Dept. of Pediatrics,
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Maryland Technology Development Corporation (TEDCO)

Curt Van Tassell
(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.

Congratulations to the 2015 MSCRF Grant Award Recipients

Pre-Clinical Grant Award:

Dr. Madhusudan Peshwa
MaxCyte, Inc.
Development of a Non-Viral, Site-Specific, Ex-Vivo Gene-Modified Cell Therapy as Treatment for CGD

Investigator Initiated Grant Awards:

Dr. Ricardo Feldman
University of Maryland, Baltimore
Using iPSC to Identify Early Markers and Biotherapeutics for GBA1-Associated Neurodegeneration

Dr. John Fisher
University of Maryland, College Park
Expansion of HSCs In a 3D Printed Bioreactor Containing MSCs as a Hematopoietic Microenvironment

Dr. Luis Garza
Johns Hopkins University- School of Medicine
Clinical Trial of Human Fibroblast Stem Cells to Convert Skin Identity and Enhance Prosthetic Use

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Mechanisms of Human Heart Precursor Renewal

Dr. Nicholas Maragakis
Johns Hopkins University- School of Medicine
Development of Imaging Biomarkers for Stem Cell Transplantation in Amyotrophic Lateral Sclerosis

Dr. Hongjun Song
Johns Hopkins University- School of Medicine
Engineering Organoids from Human Stem Cells and their Application in Safety Assessment

Exploratory Grant Awards:

Dr. Kathleen Burns
Johns Hopkins University- School of Medicine
Functional Studies of a LINE-1 (L1) Insertion in the Janus Kinase 2 (JAK2) Locus

Dr. Kan Cao
University of Maryland, College Park
In Vitro 3D Modeling of Vasculopathy in Progeria Using Patient Specific iPSCs

Dr. Ted Dawson
Johns Hopkins University- School of Medicine
Development of a Chimeric Human Mouse Model of Parkinson's Disease

Dr. Dawei Gong
University of Maryland, Baltimore
Derivation of Human Brown Adipocytes through Reprogramming for Obesity & Diabetes Research & Development

Dr. Peter Johnston
Johns Hopkins University - School of Medicine
Cell Impregnated Nanofiber Stent Sleeve for Peripheral Vascular Repair

Dr. Dara Kraitchman
Johns Hopkins University- School of Medicine
Spontaneously Occurring Spinal Cord Injury in Dogs as a Model for Studying Stem Cell Therapy

Dr. Yunqing Li
Hugo W. Moser Research Institute at Kennedy Krieger
Regulation of Oligodendrocyte Identity by MicroRNA Networks

Dr. Jiou Wang
Johns Hopkins University- School of Medicine
Pathogenic Mechanisms and Intervention Targets in Neurodegenerative Disease ALS/FTD

Dr. Zhexing Wen
Johns Hopkins University - School of Medicine
Synaptic Screening with Psychiatric Patient iPSCs Derived Neurons for Drug Discovery

Dr. Lingling Xian
Johns Hopkins University - School of Medicine
HMGA1 Chromatin Remodeling Proteins in Human Intestinal Stem Cell Homeostasis & Gut Regeneration

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

Post-Doctoral Fellowship Grant Awards:

Dr. Xitiz Chamling
Johns Hopkins University- School of Medicine
Mentor: Dr. Donald Zack
Small Molecules that Promote Differentiation of Myelinogenic Oligodendrocyte Precursor Cells

Dr. Srinivasa Raju Datla
University of Maryland, Baltimore
Mentor: Dr. Sunjay Kaushal
Allogeneic Safety Testing of c-Kit+ Cardiac Stem Cells

Dr. Jeffrey Ehmsen
Johns Hopkins University- School of Medicine
Mentor: Dr. Ahmet Hoke
Identifying New Targets to Promote Muscle Regeneration

Dr. Ileana Lorenzini
Johns Hopkins University- School of Medicine
Mentor: Dr. Rita Sattler
Role of Structural and Functional Changes of Dendritic Spines in Patient-Derived C9ORF72 iPS Neurons

Dr. Sudhish Sharma
University of Maryland, Baltimore
Mentor: Dr. Sunjay Kaushal
Characterization of the Secretome of ckit+ Human Cardiac Stem Cells for Mycardial Infarction

Dr. Hideki Uosaki
Johns Hopkins University- School of Medicine
Mentor: Dr. Chulan Kwon
MicroRNA-based Maturation of Cardiomyocytes Derived from Human Pluripotent Stem Cells

Dr. Sooyeon Yoo
Johns Hopkins University- School of Medicine
Mentor: Seth Blackshaw
Adult Hypothalamic Neurogenesis and Regulation of Feeding and Metabolism

Dr. Ki-Jun Yun
Johns Hopkins University- School of Medicine
Mentor: Dr. Hongjun Song
Modeling Neurodevelopmental Defects in Psychiatric Disorders using iPSC-Derived 3D Cerebral Organoid

Dr. Yunhua Zhu
Johns Hopkins University- School of Medicine
Mentor: Dr. Hongjun Song
Single Cell Transcriptome Analysis of Human iPS-Derived Neural Progenitors

Maryland Stem Cell Research Fund Grants - Completed

Closed: 2009 MSCRF Award:

Dr. Valina Dawson
Johns Hopkins University
Investigator Initiated Award
Neuroprotective Pathways in iPS Derived Human Neuronal Cultures

Closed: 2010 MSCRF Award:

Dr. Curt Civin
University of Maryland, Baltimore
Investigator Initiated Award
Hematopoietic Stem Cell-Enriched MicroRNAs in Human Stem Cell Differentiation and Self-Renewal

Closed: 2011 MSCRF Award:

Dr. Feyruz Rassool
University of Maryland, Baltimore
Investigator Initiated Award
Remodeling the DNA Damage Response in Induced Pluripotent Stem Cells

Closed: 2012 MSCRF Awards:

Dr. Gerald Brandacher
Johns Hopkins University
Induced Pluripotent Stem Cell (iPS) Derived Schwann Cells to Enhance Functional Recovery Following Nerve Injury and Limb Allotransplantation

Dr. Gabriel Ghiaur
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Richard Jones
Retinoic Acid (RA) Controls Self Renewal & Differentiation of Human Hematopoietic Stem Cells

Dr. Miroslaw Janowski
Johns Hopkins University
Exploratory Award
Magnet-Navigated Targeting of Myelin Producing Cells to the Stroke Via Intraventricular Route in a Large Animal Model

Dr. Minoru Ko
Elixirgen, LLC.
Investigator Initiated Award
Generating Human Induced Pluripotent Stem Cells with Less Cancer-Risk

Dr. Wenxia Song
University of Maryland College Park
Exploratory Award
In Vitro Differentiation of Human Induced Pluripotent Stem Cells into B-Cells For Modeling Human Diseases

Closed: 2013 MSCRF Awards:

Dr. Jing Fan
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Valina Dawson
PARP-1 and Histone1 Interplay and Regulate Stem Cell Differentiation after Stroke

Dr. Jeffrey Huo
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Elias Zambidis
The Role of Somatic Memory in Determining Efficient Hematopoietic Differentiation of hiPSC

Dr. Seulki Lee
Johns Hopkins University
Exploratory Award
Design of Highly Fluorinated Stem Cells for 19F-MR Imaging in Cardiac Repair

Dr. Anjali Nandal
University of Maryland, College Park
Post-Doctoral Fellowship Award
Mentor: Dr. Bhanu Telugu
Induced Pluripotent Stem Cell Derived, Immunoisolated β-cell Transplantation for Diabetes Therapy

Dr. Ludovic Zimmerlin
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Elias Zambidis
Genetic Correction of Sickle Cell Disease Human iPSC Converted to a Murine ESC-Like State

Closed: 2014 MSCRF Awards:

Dr. Ian Martin
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Ted Dawson
Identifying Targets of LRRK2 Translational Regulation in Parkinson's Disease Patient Human Dopamine

Dr. Jun Wang
Phycin, LLC.
Exploratory Award
Recombinant Growth Factors from Algae and their Application in Human Pluripotent Stem Cell Research

> “... **Stem cell research** could lead to new treatments and cures for the many Americans afflicted with life-threatening and debilitating diseases.”
>
> *- Rep. Ron Kind-*

2015

Pre-Clinical Research Grant Award:

Madhusudan Peshwa, Ph.D.
MaxCyte, Inc.
Award Amount: $475,000
Disease Target: Chronic Granulomatous Disease (CGD)

Development of a Non-Viral, Site-Specific, Ex-Vivo Gene-Modified Cell Therapy as Treatment for CGD

MaxCyte is world-leader in clinical and commercial development of engineered, potency-enhanced cell therapy products using a manufacturing process with a cGMP and regulatory compliant, automated, cost-effective, closed, scalable system. Using mRNA to perform site-specific genome editing in primary cells, we have entirely eliminated the need for use of viral vectors in gene therapy and successfully translated 4 partnered programs into US FDA IND cleared human clinical trials. More recently our preliminary study demonstrated that efficient correction of the mutated gene in both CGD-patient lymphocytes and CGD-patient hematopoietic stem cells can be achieved, leading to an unprecedented efficacy in reversing a clinically relevant level of 10% and 6.3% of disease cells into cured heath cells in CGD-patient lymphocytes and adult hematopoietic stem cells respectively. These results provide motivation to use CGD as an example to further evaluate the application of such approaches to enabling development of potential cures for untreatable genetic diseases. represents an unmet medical need and a significant cost to our healthcare system and burden on society.

In this study, specifically we propose to correct the mutation in hematopoietic stem cells in CGD, as a proof-of-concept, based on our initial observation. CGD is a group of rare hereditary diseases in which phagocytes do not produce reactive oxygen compounds (most importantly, the superoxide radical) used to kill certain pathogens. CGD is a rare disease affecting about 1 in 200,000 people in the United States, with about 20 new cases diagnosed each year. Management of CGD involves early diagnosis, patient education and antibiotics for prophylaxis and treatment of infections. The morbidity of recurrent infections and inflammation is a major issue, with rates of infection around 0.3 per year. CGD is thus an example of a genetic disease where there is no clinically meaningful therapy; and thus represents an unmet medical need and a significant cost to our healthcare system and burden on society.

We will use the MaxCyte's regulatory compliant, closed manufacturing platform to deliver messenger RNA molecules that surgically correct mutated nucleotides in hematopoietic stem cells (HSC) of CGD patients and replace them with correct nucleotides to revert the mutated (disease causing gene) into a normal gene. If our results demonstrate success in correcting the disease gene to normal state in 5-15%, hypothesized to be significant for clinically meaningful benefit – this would in effect validate the platform as potentially curative therapy for expansion of the same approach to cure multiple other diseases where known mutations in normal gene sequences are associated with diseases.

2015

Investigator Initiated Research Grant Awards

Ricardo Feldman, Ph.D.
University of Maryland, Baltimore
Award Amount: $655,500
Disease Target: Gaucher Disease & Parkinson's Disease

Using iPSC to Identify Early Markers & Biotherapeutics for GBA1-Associated Neurodegeneration

Mutations in the GBA1 gene cause Gaucher disease (GD). GBA1 encodes the lysosomal enzyme glucocerebrosidase (GCase). 7% of individuals with Parkinson's disease (PD) also have mutations in the GBA1 gene, making it the most common genetic risk factor for PD. When both copies of GCase have severe mutations, there are serious neurological symptoms that are fatal within 2 years of life. When the disease is subacute, children and adolescents show neurodegeneration and manifestations of Parkinson's disease. Individuals that inherit only one mutated GCase gene are also at increased risk of developing PD. PD is characterized by a loss of neurons that produce the neurotransmitter dopamine (dopaminergic neurons). The mechanisms by which GCase deficiency lead to neurodegeneration, and the connection between GD and PD have remained obscure because of the limited availability of neuronal tissue from affected patients. To overcome these limitations we generated induced pluripotent stem cells (iPSC) from patients harboring mutations in GCase. We found that when iPSC with GCase mutations were differentiated to neurons there was a 50-95% selective loss of dopaminergic neurons, compared to iPSC controls. We also found that the extent of the neuronal loss depended on how inactivating the mutations were. Further analysis showed that mutant GCase deregulated a developmental pathway that specifies dopaminergic differentiation (Wnt/β-catenin pathway). This resulted in a loss in the production of key transcription factors required for dopaminergic differentiation. Our results provide a critical insight about the molecular mechanisms that lead to dopaminergic loss and PD. We hypothesize that mutant GCase deregulates the Wnt/β-catenin pathway, interfering with the induction of dopaminergic-specific transcription factors. We also hypothesize that this developmental defect depends on the severity of the GCase mutation and gene dosage. In this application we will elucidate the mechanisms involved, and will test the efficacy of new drugs to reverse the loss of dopaminergic neurons. In Aim 1, we will use gene-editing techniques to determine whether severity of the mutation, and the transition from single to double GBA1 mutations, are reflected in the extent of dopaminergic loss. This has never been done and is important for the choice of the best cellular reagents to use for drug discovery. In Aim 2, we will identify the mechanisms by which GCase deficiency leads to selective loss of dopaminergic neurons. Our focus will be on the Wnt/β-catenin pathway. Importantly, there are very well known potent inducers of Wnt and related pathways. In this application we will test whether these pharmacological agents can bypass the developmental block caused by mutant GCase, and restore dopaminergic differentiation. In Aim 3, we will test the therapeutic efficacy of new drugs recently identified that stabilize the mutant GCase. As patients with PD where GCase is not mutated also have decreased expression of GCase, the proposed work may lead to diagnostics and treatments for other forms of PD as well. The work proposed is based on a novel concept, it represents a radically different approach to identify new therapies for Gaucher and PD, and it will lead to clinical trials.

John Fisher, Ph.D.
University of Maryland, College Park
Award Amount: $655,500
Disease Target: Aplastic Anemia & Cancer

Expansion of HSCs In a 3D Printed Bioreactor Containing MSCs as a Hematopoietic Microenvironment

Limited growth of stem cells outside of the body is one major roadblock to their widespread use in regenerative medicine. In particular, research and clinical application of both mesenchymal stem cells (MSCs, which form bone, cartilage, muscle, and other cell types such as stromal cells which nurture HSCs in the bone marrow) and hematopoietic (blood-forming) stem cells (HSCs) would benefit from technology that provides a platform for their expansion and growth while maintaining their ability to self-renew. Each aim of this proposal addresses this problem by applying a novel, 3D printed growth platform to the expansion of (1) MSCs, (2) HSCs, and (3) a combination of the MSCs and HSCs. The underlying idea is that we can recapitulate some of the important physical cues of the native stem cell niche, and that recapitulating this microenvironment will enhance the expansion of MSCs and HSCs with self-renewal capacity. Successful completion of this project would demonstrate our ability to custom fabricate a platform for the growth of these stem cell lines in a laboratory setting. By enabling researchers to grow larger numbers of cells than they typically would via traditional methods, more investigative work into understanding these stem cells could take place. Furthermore, the utilization of stem cells for regenerative medicine applications could be expedited.

Luis Garza, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $640,000
Disease Target: Prosethetic Skin Breakdown & Amputations

Clinical Trial of Human Fibroblast Stem Cells to Convert Skin Identity & Enhance Prosthetic Use

Clinical trial of human fibroblast stem cells to convert skin identity and enhance prosthetic use amputations are increasing because of diabetes and trauma in our population. Major improvements are underway in the modernization of prosthetics to help outfit amputees with new limbs. However these improvements in design are limited by the human interface. Skin breakdown and pain at the stump site commonly limit the use of prosthetics. Our hand and feet (volar) skin are frequent points of interaction with our environment, but do not develop skin breakdown because of features like a thicker top layer of epidermis, and stronger structural components. This proposal investigates a therapy to convert the weight bearing skin characteristics normally present on palms and soles (volar skin) to the stump site as a means to enhance performance among modern prosthesis. We propose to use site-specific fibroblast stem cells to convert this skin identity. This research promises to directly improve the quality of life of amputees. By creating volar-like weight bearing skin at stump sites, this proposal aims to make the placement of a prosthetic at the leg no different from the placement of a shoe on a foot. In the same way that the soles of the feet do not normally have frictional injuries from the use of socks and shoes, and yet bear the weight of a person, we hope that the converting stump skin to volar skin should similarly yield consistent and unencumbered use of a prosthetic—for both arm and leg amputees. We are already approved by the FDA and our IRB to investigate this therapy. Not only will this therapy help amputees, given its status in clinical testing it will be soon ready for commercialization and thus aid the economy of Maryland. The PI has already talked with several venture capital companies about their interest in this technology.

David Kaetzel, Ph.D.
University of Maryland, Baltimore
Award Amount: $655,500
Disease Target: Osteoporosis

NME1 as a Master Regulator of Human Mesenchymal Stem Cell Phenotype

Stem cells hold great promise for regenerative medicine in a wide variety of human diseases, injuries and other conditions. Mesenchymal stem cells (MSCs) have become a strong focus for development of stem cell therapies, as they can be readily harvested and expanded in culture from human tissues such as bone marrow, blood and such tissues as bone, cartilage, ligaments and tendons. MSCs are potentially useful in diseased or damaged tissues because they can be induced to change (or differentiate) into more specialized cells (e.g. cartilage, fat and bone cells) in those sites to promote healing and regeneration. Importantly, MSCs possess two distinct advantages over embryonic stem cells (ESCs) for therapeutic application: 1) they can be derived from discarded human tissues and not human embryos, and 2) they do not form tumors, which is a hallmark of ESCs. One key limitation, however, is a current inability to stabilize MSCs during to acquire the large numbers needed for therapies, while retaining their full ability to regenerate tissues. A very recent study has demonstrated that a protein, NME1, possesses a powerful ability to maintain the quality of human ESCs (Smagghe et al., 2013). NME proteins are indeed required for maintenance of the MSC as well, and our recent preliminary data have demonstrated potent growth factor activity of NME1. The primary working hypothesis of this proposal is that NME1 and/or the related protein NME2, will maintain proliferation, differentiation capacity (“pluripotency”), and genomic stability of cultured human MSCs during their expansion. This project has been designed to advance the stated goals of the MSCRF. It will broaden our current knowledge of human stem cell biology by elucidating the role of NME1 and NME2 in maintenance of MSCs in their undifferentiated and pluripotent condition. Importantly, the studies are designed to optimize the use of NME proteins as single agent additions to defined culture media formulations for enhancing yields and quality of T-MSCs. The ultimate goal of the project is to generate T-MSCs in a manner compliant with Good Manufacturing Practice (GMP) for regenerative medicine approaches to a wide variety of diseases, injury settings and other conditions. We anticipate the project will provide the necessary strong foundation to accomplish this goal, in future cooperative efforts with collaborators in the clinical and biotechnology settings.

Vassilis Koliatsos, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $655,500
Disease Target: Traumatic Brain Injury

Stem Cell Therapies for Traumatic Brain Injury: Contusions and Axonal Injuries

A common devastating type of traumatic brain injury (TBI) is diffuse axonal injury. This is widespread damage to axons, i.e. specialized processes of nerve cells (neurons) to communicate with each other. These pathologies are encountered in motor vehicle crashes, collision sports, and military operations and they impair higher nervous system functions. Diffuse axonal injury is associated with high mortality in the acute phase and causes severe chronic neurological and psychiatric disability in survivors. Unfortunately, we do not have satisfactory treatments for this condition. Regenerative medicine is perhaps the best idea for treatment in the current state of the field. Diffuse axonal injury is a problem of disconnection of brain regions, so the idea is to make new connections with additional brain cells. In the present application, we propose a cell therapy for diffuse axonal injury, motivated by enthusiasm from previous findings in our laboratory. In our previous work we had shown that human stem cells committed to making nerve cells, named “neuronal precursors” (NPs) can become fully differentiated neurons and then begin to engage in synaptic communication with other neurons in the brain. In our current project we expect that human NPs will mature and replace damaged nerve cells and axons in the injured brain. We also expect that human stem cells that are cultured such as to mature into oligodendrocytes, i.e. cells that wrap themselves around axons and facilitate conduction of nerve signals, will protect damaged nerve cells or help them regenerate axons. We test these ideas in a rat model of diffuse axonal injury and select to focus on the motor system, which always becomes affected in models of diffuse axonal injury. We transplant stem cells that make neurons with or without stem cells that make oligodendrocytes and see whether such transplants recreate new functional motor circuits. The physiological activity of new circuits is tested with modern molecular techniques involving light-sensitive ion channels and the use of light shone against transplanted cells to turn them on or off. We also ask if the new physiologically active motor circuits formed by such cells help injured animals regain motor function. The idea is that, if the new circuits formed by these cells are capable of restoring motor behavior in these animals, then turning these transplanted cells off should abolish the behavioral benefits of transplants. Based on our previous experience and our partnership with other experts in our medical school community we hope that we will achieve our goal of partial restoration of brain circuits and functions in injured animals with human stem cells. The use of human stem cells in appropriate animal models that recapitulate features of a major form of TBI and the testing of functional and behavioral outcomes makes this project very applicable to future clinical trials of cell therapies in TBI patients.

Chulan Kwon, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $655,500
Disease Target: Heart Disease

Mechanisms of Human Heart Precursor Renewal

Congenital heart disease remains the most frequent type of birth defect and the leading cause of birth defect-related deaths. Cells giving rise to the heart, so called cardiac progenitor cell (CPCs), serve as building blocks to form the heart during fetal development and thus abnormal CPC development is closely associated with congenital heart disease. Thus, it is crucial to understand the biology of CPCs and their environment. Our lab discovered a CPC population that can multiply without becoming heart cells in a cellular environment known as the second pharyngeal arch and this proposal aims to understand genetic factors that control the event. This knowledge will provide first insights into how cellular and environmental factors affecting CPC multiplication can lead to congenital heart disease, which may allow us to uncover new diagnostic, preventive or even therapeutic options. In addition, the knowledge gained from this work may allow us to produce a large number of cardiac muscle cells for patient-specific disease modeling and transplantation to treat heart disease.

Nicholas Maragakis, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $655,353
Disease Target: Amyotrophic Lateral Sclerosis

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

ALS, also known as Lou Gehrig's Disease, affects roughly 5,000 people in the U.S. per year and as many as 20,000 Americans currently suffer from this disease. ALS is a neurodegenerative disease characterized by the progressive deterioration and loss of both upper and lower motor neurons resulting in muscle atrophy, progressive weakness, and ultimately death. The length of survival in most subject populations that have been evaluated is 2 to 5 years with death often the result of respiratory failure due to loss of cervical motor neuron control of diaphragmatic function. The rationale of using a cellular therapeutic to provide healthy astrocytes to treat ALS subjects is supported by numerous studies confirming the role of diseased astrocytes in ALS pathogenesis, and benefits of healthy astrocytes in preserving motor neuron function. This leads to the hypothesis that the introduction of healthy astroglia via transplantation of glial progenitors (GRP: glial restricted progenitor) is a promising therapeutic approach for slowing and/or halting disease course. Therefore, human Glial Restricted Progenitor cells (Q-Cells), are being studied for their potential in providing neuroprotection in ALS. The development of Q-Cells as a cellular therapeutic for ALS has already seen significant progress. We have already performed the preclinical studies including biodistribution and tumorigenicity studies in rodent and minipig models for our planned clinical trial using Q-Cells in ALS subjects. Our data suggest that Q-Cells do not show any evidence of forming tumors nor do they migrate in unwanted regions outside the brain and spinal cord. The data from minipig models suggest that the device for delivering these cells to the spinal cords of subjects is safe and that the strategy for immune-suppression results in robust cell survival. These studies have provided the foundation for the design of a clinical trial framework for a Phase I/IIa study in ALS subjects. However, one of the fundamental limitations in assessing how well cells might work after transplantation into ALS patients is the capacity for monitoring cell survival and migration non-invasively. The brain and spinal cord are particularly challenging, as they are not accessible to biopsy for analyzing cell survival. Another significant challenge to stem cell therapeutics in neurological disease is the availability of a biomarker (a marker of disease activity) that can indicate cell-specific effects on the target. Therefore, in this study, we will use MRI-based techniques to examine cell survival and migration over time following the transplantation fluorine-labeled Q-Cells into rodent models of ALS. In the same animals, we will also examine a biomarker of tissue glutamate (a potential motor nerve "toxin") using the MRI-based GluCEST imaging strategy. Since the clearance of tissue glutamate is a proposed hypothesis for Q-Cell neuroprotection, this will provide important information for determining how well these cells are protecting motor neurons from dying. Taken together, this study will provide an important next-step in human stem cell clinical trial design as techniques for non-invasively studying cell survival and migration over time will be explored and the effect of this cell transplantation strategy on a disease-relevant biomarker will be assessed. These steps are necessary in helping to understand the best strategies for delivering cells to the spinal cord where the disease is most active and also to understand how these cells might be working in patients with ALS.

Hongjun Song, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $655,500
Disease Target: Multiple

Engineering Mini-Brains from Human Stem Cells and Their Application in Safety Assessment

Groundbreaking discoveries are continuously made in stem cell research, such as turning human skin cells into stem cells with the capacity to become any cell type in the body. Traditionally, studies of stem cells are performed in monolayer cultures for better control, but this approach cannot model the assembly and organization of different cell types observed in organs. One major recent advance is a method for generating three-dimensional (3D) cultures that produce structures resembling whole developing organs from human stem cells, named organoids. Human cell-based organoids provide a unique opportunity to model organ development in a system that is remarkably similar to human organogenesis in vivo, which is not typically accessible to experimentation and drug testing. But before the organoid technology can be widely applied for modeling human development and diseases, drug testing and future organ replacement, a number of roadblocks need to be cleared. First, the current system is not affordable for the majority of investigators. The tremendous cost is partly due to the need to use a large volume of very expensive reagents to culture these cells in a spinning bioreactor. As a consequence, it is also cost prohibitive to test different conditions, which is essential for optimization of the technology and for large-scale drug screening. Second, the current technology for generating cerebral organoid (mini-brains) is largely based on cell self-organization with little external control, leading to large sample to sample variations that limit its use for quantitative studies. The current project aims to address and overcome these major roadblocks. We will use 3D printing technology to develop a series of miniaturized spinning bioreactors for culturing cerebral organoids from human stem cells under different conditions with 100 fold reduction in cost. Second, we will develop methods for reproducibly generating uniform and more mature forebrain organoids from human stem cells. Finally, we will exploit our new human organoid technology to develop a platform for assessing chemical toxicity. We expect our device will significantly accelerate progress in the whole field, making it affordable to many investigators and for large-scale applications. We also envision our forebrain organoid technology will have versatile applications for the modeling of normal and diseased human brain development. Demonstration of the efficacy of our novel screening platform would be of interest to the pharmaceutical industry, as well as Environmental Protection and Regulatory Agencies.

> Without a doubt, **stem cell research** will lead to the dramatic improvement in the human condition and will benefit millions of people.
>
> *-Eli Broad-*

2015

Exploratory Research Grant Awards:

Kathleen Burns, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $200,000
Disease Target: Myeloproliferative Neoplasms;
Diseases Treatable by Bone Marrow Transplant

Functional Studies of a LINE-1 (L1) Insertion in the Janus Kinase 2 (JAK2) Locus

Myeloproliferative neoplasms (MPNs) are a group of blood diseases, with over production of blood cells, and can be transformed to leukemia. JAK2V617F mutation is a nucleotide change in JAK2 gene, which has been discovered as the most prevalent mutation in MPNs. Studies have demonstrated that people who have specific DNA variants in a specific region including JAK2, referred to as '46/1', are predisposed to acquire JAK2V617F mutation and develop MPNs. Which variants in this region are responsible for the risk is still unknown. Our genome is rich of repetitive DNA recognizable as retrotransposon derived by mobile DNAs, whose function are little known. We have identified a novel retrotransposon insertion called L1 LINE (long interspersed elements) in JAK2, which resides in the important 46/1 risk region. Our overarching hypothesis is that this L1 structural variant is a key determinant of JAK2 gene expression levels with implications for differentiation determination of blood cells, thus to provide a selective advantage for JAK2V617F clones. Our specific objectives are to modify the human Induced pluripotent stem cell (iPSC) lines derived from MPN patients with JAK2 normal allele and JAK2V617F mutation allele and obtain the modified iPSC lines for presence/absence of the JAK2 L1 insertion using precise genome editing system; and next to assess effects of the L1 on the differentiation potential of iPSCs and on JAK2 expression in iPSCs and derived blood cells during differentiation in vitro. The proposed studies are expected to inform how stem cell models can be used to study roles of common genetic structural variants, provide practical insights on the blood cell developmental potential of iPSCs, promote our understanding of the biology of MPNs, and suggest how the genetic background of a person influences their blood cell differentiation tendencies and risks for leukemia. This research will provide information for potential studies to evaluate and decrease the risk of leukemia transformation by gene-modified stem cell therapies.

Kan Cao, Ph.D.
University of Maryland, College Park
Award Amount: $218,500
Disease Target: Progeria & Cardiovascular Disorders

In Vitro 3D Modeling of Vasculopathy in Progeria Using Patient Specific iPSCs

Hutchinson-Gilford Progeria syndrome (HGPS), although rare, has devastating consequences to the affected children. Those with HGPS undergo accelerated aging and have an average life expectancy of just 13.4 years. Symptoms can include a pronounced forehead, short stature, receding mandible, extreme lipodystrophy, severe osteoporosis and a high incidence of bone fracture. In adition, patients with HGPS suffer from accelerated organ degeneration, and death almost always results from coronary artery disease or stroke. Previous research has shown massive loss of smooth muscle cells (SMCs) in large vessels in HGPS patients and animal models, suggesting a possible link between this phenotype and the deadly cardiovascular malfunction in HGPS patients. Recent study conducted in our group has uncovered a mechanistic model that can explain this SMC loss phenotype in HGPS, using patient iPSC derived SMCs. To further study this mechanistic model, here, we propose to build and characterize a 3D tissue engineered blood vessel (TEBV) system using HGPS iPSC derived SMCs (Aim1), and to analyze our mechanistic model in the TEBV system under conditions mimicking in vivo environment (Aim2). This study will be conducted in collaboration with Dr. George Truskey at Duke University, a leading expert in blood vessel engineering. We expect that this 3D TEBV system of HGPS will be of great importance for future drug screening and therapeutic development for HGPS and age-related cardiovascular diseases.

Ted Dawson, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,500
Disease Target: Parkinson's Disease

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

This project will optimize conditions for creating humanized mouse models of Parkinson's disease (PD). We will use state-of-the-art technology to generate ground state naïve human embryonic and inducible pluripotent stem cells. These ground state naïve human embryonic and inducible pluripotent stem cells will be injected into genetically engineered mice that do not develop dopamine neurons, which will create an environment that will permit the integration of human dopamine neurons in the living mouse brain. This has the potential to revolutionize the study of the underlying pathogenesis of a variety of human disorders. PD is the most common movement disorder that is due, in part, to the preferential loss of dopamine (DA) neurons. The relative selective degeneration of DA neurons makes PD a particularly attractive human neurodegenerative disease to establish a humanized mouse model of neurodegeneration. The mechanisms underlying the development of DA neurons is well established and there are robust protocols that can be used to study the function of these humanized DA neurons in the intact mouse brain. These set of investigations and aims have the potential to transform the study of neurodegenerative disease in general and in particular the study and treatment of PD by providing new molecular insights into the pathogenesis of PD. It is anticipated that these humanized models will serve as an in vivo model for drug development and it will lead to the discovery of new biochemical and/or molecular markers that could be ultimately used as targets to prevent the degeneration of DA neurons in PD.

Dawei Gong, Ph.D.
University of Maryland, Baltimore
Award Amount: $218,500
Disease Target: Obesity & Diabetes

Derivation of Human Brown Adipocytes through Reprogramming for Obesity & Diabetes Research & Development

Obesity is prevalent in the US with one-third of the US population classified as being obese and is a major risk factor for diabetes and cardiovascular diseases. Although dieting and exercise are an effective prevention and treatment for obesity, weight loss through lifestyle change alone is difficult to sustain for a majority of obese people. Thus, effective treatment has been sought to curb the epidemics of the disease. Fat tissues can be divided into the white and brown adipose tissues. White adipose tissue is for storing fat (obesity is caused by too much fat storage in the white adipose tissue) and the brown adipose tissue is specialized to burn calories to generate body heat in rodents and newborn humans. Recent studies show that adult humans do possess the brown fat tissue in an indistinctive manner. Thus, obesity researchers are seeking to combat the obesity epidemic by stimulating browning activity of white adipocytes or increasing the amount of brown adipose tissue. In this application, we propose to directly convert human white adipocytes into brown adipocytes through cell reprogramming and to transplant the reprogrammed brown adipocytes in mice to determine their anti-obesity activity in the animal. We will also test, in human fat cells, the browning activity of two small molecules obtained through a high-throughput screening of a library of 5,000 FDA approved proto-drug library. Collectively, we expect a success of the proposed studies will help to lead to a new therapeutics for obesity and its associated disease.

Peter Johnston, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,237
Disease Target: CLI & Peripheral Vascular Disease

Cell Impregnated Nanofiber Stent Sleeve for Peripheral Vascular Repair

Critical limb ischemia (CLI), the lack of blood flow to a leg or arm to the point that it causes the limb tissue to die, is the final stage of peripheral artery disease and is associated with poor outcomes: up to 50% of patients require amputation and 25% die within a year of the diagnosis. These bleak statistics drive the search for novel therapies such as stem cells, which have the potential to stimulate blood vessel growth and restore blood flow to dying tissue. To date stem cells have been given in clinical trials to patients with CLI, but the results have been mixed. One reason is that stem cells delivered to damaged or dying tissues remain there for only a short period of time. In addition there is evidence that the beneficial effects of stem cells result primarily from the release of substances called paracrine factors (PFs) that stimulate the body's own repair mechanisms. In the case of CLI, these PFs promote blood vessel formation and trigger tissue regeneration. In theory, if a method could be developed to allow for delivery of these beneficial PFs to an ischemic limb over a sustained period, the limited effects seen in clinical trials could be substantially improved upon. These concepts have driven our interest in developing a novel method to deliver stem cell therapy using a device designed to improve stem cell survival and retention in the body while allowing free release of their PFs. The device involves weaving nanofibers to generate a biocompatible matrix in which stem cells can be incorporated and continue to grow and thrive. The matrix prevents the stem cells it contains from leaving, but allows free release of the beneficial PFs produced by the cells. The matrix also prevents other cells from entering, especially immune cells, which could trigger rejection. For delivery to the body the nanofiber matrix is applied to a vascular stent identical to those used to treat blockages in the arteries of the arms, legs, and heart. In this configuration the nanofiber matrix forms a "sleeve" around the stent, and the device is called a „Cell-Impregnated Nanofiber Stent Sleeve" or "CINSS". When delivered to an artery the CINSS will provide a safe haven from which contained cells can release their beneficial PFs to the injured tissues downstream from the stent. Preliminary experiments have shown that adult human stem cells readily incorporate and grow in the CINSS, and retain the ability to release beneficial PFs that can generate blood vessel formation in bench top experiments. Further testing has shown that the CINSS can be implanted in the artery of research animals without immune rejection or other adverse effect. We now intend to study whether the CINSS can have a beneficial effect in an animal model of CLI. If this approach is shown to be effective, the work done here would allow for rapid translation to clinical studies with the ultimate goal of improving outcomes for patients with CLI.

Dara Kraitchman, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,500
Disease Target: Spinal Cord Injury

Spontaneously Occurring Spinal Cord Injury in Dogs as a Model for Studying Stem Cell Therapy

Over 250,000 Americans are living with neurological dysfunction due to spinal cord injury (SCI), which occurs most frequently due to motor vehicle accidents and falls. While beneficial effects of stem and progenitor cell therapy has been shown in animal models of SCI, these therapies have been slow to translate to treatment in patients. Because these animal models create SCI in an invasive manner using a surgical approach, they poorly mimic traumatic SCI in humans. Whereas, the benefit of stem cell therapies may be largely due to their anti-inflammatory properties as well as regeneration of the spinal cord, most SCI animal models are performed in mice and rats, whose immune system is vastly different than people or is suppressed to enable the study of human stem or progenitor cells. Moreover, non-invasive methods to study SCI, such as magnetic resonance imaging (MRI), are performed on specialized small animal imaging units, which further impedes translation of techniques to people. Thus, many clinical trials in patients fail to show the large beneficial effect of stem cells that has been reported in animal models.The current proposal seeks to use a more realistic model of SCI by using naturally occurring disease that occurs in client-owned pets. Intervertebral Disc Disease (IVDD), which results in spinal cord compression due to disc protrusion or extrusion is fairly common in dogs and — similar to SCI in humans— is a medical-surgical emergency. The clinical signs in dogs of IVDD ranging from loss of sensation to hind limb paralysis and loss of bowel and bladder function and are similar to SCI in human patients. IVDD is diagnosed using MRI, and surgery to remove the affected disc and remove pressure on the spinal cord is performed. Recovery of function after surgery is dependent on the degree of dysfunction and length of clinical signs, but usually some degree of dysfunction remains as in SCI patients. Thus, stem and neural progenitor cell delivery during back surgery in dogs provides a more faithful representation of SCI in people with which to test new emerging therapies for safety/efficacy with the added benefit that it might help dogs too. The goal of the current proposal is to change the classic paradigm of animal testing followed by patient clinical trials by testing therapies in pets with naturally occurring diseases to develop improved treatments. Johns Hopkins University established the Center for Image to assist with developing promising new therapies. In the current study, we seek to determine whether stem cells combined with a cell that is committed to a nerve cell fate called neural restricted progenitors (NRPs) is better than surgery alone. Because IVDD surgery must be performed promptly after the first signs of spinal cord compression, there is not sufficient time to expand stem cells prior to delivery at surgery. Thus, we will need to use stem cells from a donor that are placed in a specialized capsules, developed at Johns Hopkins, to prevent rejection. First, we will test the safety of using fat-derived stem cells in a capsules in normal laboratory dogs. Then, we will initiate a stem cell clinical trial in dogs with IVDD where these capsules are delivered during surgery to the spine. In preparation for a more sophisticated trial, we will also test the safety of NRPs combined with fat-derived stem cells in laboratory dogs. Ultimately, we believe that this study will form the basis of testing promising stem and progenitor cell therapies in dogs and humans.

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

Regulation of Oligodendrocyte Identity by MicroRNA Networks

Cell transplantation therapy using oligodendrocyte progenitor cells (OPCs) holds great promise for treating central nervous system (CNS) diseases in which oligodendrocyte injury and death leads to demyelination (loss of myelin) or dysmyelination (abnormal myelination). 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 a mixture population of oligodendrocytes (OLs) and astrocytes. Optimized differentiation strategies for generating functional OLs in sufficient numbers and purity for cell transplantation are urgently needed. The generation of an OL population requires the initiation of a specific gene expression program. In this process, OL transcriptional networks are induced, and neuronal and astrocyte lineage transcriptional networks are repressed. MicroRNAs (miRNAs, small noncoding RNAs) as negative regulators of gene expression play critical roles in OL development. We have recently found that miR-148a expression regulates OL differentiation by inhibiting DNA methyl-transferases (DNMTs) genes, proneuronal differentiation gene (NeuroD1) and pluripotency genes. Using non-viral biodegradable polymeric nanoparticle-based transcription factors and miRNA delivery technology, we plan to determine if expression of miR-148a will enhance and drive hiPSCs into the OL lineage fate when combined with OL lineage transcription factors Sox10 and Olig2, both of which are implicated in the transcription control of OL development. In addition, we will verify the functionality (myelination) and safety (no tumor formation) of the newly generated hiPSCs-derived iOPCs using mouse models. Successful completion of these experiments will enable us to generate sufficient, homogenous, and safe patient-specific OPCs with myelination capabilities that can be used for cell replacement therapy for treatment of spinal cord injury and other CNS disorders.

Jiou Wang, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,500
Disease Target: ALS, Dementia, and Alzheimer's Diseases

Pathogenic Mechanisms and Intervention Targets in Neurodegenerative Disease ALS/FTD

With a doubling of the average human lifespan over the last century, neurodegenerative diseases have become a major aging-related public health challenge in the US and many other countries. Unfortunately, no curative treatments exist for these debilitating conditions, which are also poorly understood. Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is a progressive neurodegenerative disease characterized by the degeneration of motor neurons. Unfortunately, the mechanisms underlying this motor neuron degenerative disease remain poorly understood. The work outlined in this proposal is designed to investigate the basic pathogenic mechanisms underlying expansion of the hexanucleotide repeat (HRE) in C9orf72, a genetic abnormity that has recently been found to cause the most common form of ALS. In 2011, the hexanucleotide repeat expansion (HRE), in a noncoding region of C9orf72 was linked to the neurodegenerative disease ALS and frontotemporal dementia (FTD). Aside from being the most common cause of ALS, the C9orf72 HRE also represents the most common genetic cause of FTD, which is characterized by degeneration of the frontal and temporal lobes of the brain and is the second most common type of dementia for people older than 65. Moreover, the C9orf72 HRE also contributes to other neurological conditions, including Alzheimer's disease, Huntington's disease, multiple system atrophy, depressive pseudodementia, bipolar disorder, and schizophrenia. With the ever-expanding spectrum of C9orf72-associated diseases, the mechanism underlying the pathogenesis linked with the C9orf72 HRE remains poorly understood. Using stem cells derived from patients carrying the C9orf72 mutation, we recently discovered a molecular mechanism through which the C9orf72 HRE causes the disease pathology through unconventional structures formed by the repeat at the DNA and RNA levels. We propose that these unconventional structures initiate a cascade of pathogenic events and eventually wreak havoc in patient brains. In fact, the stem cell is best model system developed thus far to study the disease mechanism of the C9orf72 mutation in a patient-relevant system. Here in this project we will utilize the stem cell system to dissect the molecular basis of this devastating disease in search of it fundamental cause. Furthermore, we propose a new strategy to develop therapeutic agents that can provide intervention at the root of the disease and be translated to clinical use quickly. If successful, our work may lay a foundation for further studies of the most common form of ALS/FTD, and provides new targets to counter the toxic conformations of the repeats as an effective intervention at the start of the pathogenic cascade.

Zhexing Wen, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,500
Disease Target: Mental Disorders

Synaptic Screening with Psychiatric Patient iPSCs-Derived Neurons for Drug Discovery

Severe psychiatric illnesses, such as schizophrenia and major depression, are chronic and complicated neurological diseases which affect a large portion of the world's population. However, the causes of these disorders are still poorly understood and effective treatments are very limited, due to the lack of disease relevant and human relevant models. Thus, development of rational therapeutics based on understanding of the etiology and pathogenesis of the disease is imperative. Human induced-pluripotent stem cells (iPSCs), which carry the same genetic information of the donor individuals, pave the way to understand the molecular basis of human diseases and for drug discovery in more tractable experimental systems. In this proposal, we will perform a non-biased high-throughput screening for drug development by using iPSC-derived human neurons (the structural and functional unit of the nervous system) from a pedigree (Pedigree H) with a mutation in the DISC1 (Disrupted-in-schizophrenia 1) gene, a well-supported susceptibility gene for major psychiatric disorders. Information from one neuron flows to another neuron across the short gap between cells, called the synapse. Dysregulated neurodevelopment with altered structural and functional connectivity is believed to underlie many neuropsychiatric disorders and "a disease of synapses" is the major hypothesis for the biological basis of schizophrenia. By generation of non-integrated iPSC lines from members of Pedigree H and further differentiation of these iPSCs into disease relevant cell types, the cortical neurons, we have demonstrated that mutant DISC1 causes aberrant synaptic formation and synaptic vesicle release deficits. Importantly, by taking a mechanism-guided approach we found that treatment of rolipram, a PDE4 inhibitor and an antidepressant, largely rescued synaptic defects in DISCI mutant neurons, suggesting that synaptic defects in DISC1 mutant neurons can be rescued by small molecules. Our proof-of-principle study thus not only reveal that a psychiatric disorder-relevant mutation causes synapse deficits, but also provide an opportunity to develop a phenotypic high-throughput screening for candidate molecules that can ameliorate the disease relevant synaptic defects in patient neurons. In this project, to identify more efficient therapeutic compounds for treating human psychiatric diseases, we will develop a nonbiased iPSC-based synaptic screen for drugs that rescue the synaptic phenotypes in human DISC1 mutant neurons. Our novel drug screening platform therefore may lead to new therapeutic strategies for these devastating diseases that affect millions of people worldwide.

Lingling Xian, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $218,500
Disease Target: Regenerative Medicine, Gastrointestinal Disease

HMGA1 Chromatin Remodeling Proteins in Human Intestinal Stem Cell Homeostasis & Gut Regeneration

This proposal is directed at developing stem cell technology to derive gut-like tissue for use in regenerative medicine. Gut stem cells (also called intestinal stem cells or ISCs) are responsible for maintaining normal gut integrity and function throughout life. In fact, these remarkable cells replace the entire gut lining every 3-5 days in order to maintain an effective barrier and protect the gut from bacteria, other germs, and toxins. We are studying factors important for stem cell function and our focus is the high-mobility group A1 (HMGA1) gene. In work funded by the Maryland Stem Cell Research Fund, our laboratory discovered that HMGA1 functions as a key "molecular switch" by turning on genes important for stem cell function in embryonic stem cells during development. We also found that HMGA1 is required for reprogramming adult cells (such as skin or blood cells) into stem-like cells, called induced pluripotent stem cells or iPSCs. In genetically engineered mice (called transgenic mice) we discovered that HMGA1 causes a thickened intestinal lining, polyps, and expansion in the number of ISCs throughout the gut. These mice also have an increase in specialized cells, called Paneth cells, which secrete growth factors to help ISCs survive and generate new gut tissue. Using innovative models developed in our laboratory, we have begun to determine more precisely how HMGA1 enhances ISC function. We generated 3-dimensional, self-organizing intestinal tissue called "organoids" or "miniguts" by growing gut tissue from our transgenic mice under specialized conditions. Strikingly, these organoids or "miniguts" grow much more rapidly and generate more gut tissue when they express high levels of HMGA1. In preliminary experiments with human colon biopsies, we are developing innovative technology to increase HMGA1 expression in the ISCs. Similar to our mouse studies, we discovered that HMGA1 also enhances growth and development of "miniguts" from human colon biopsies. Based on these discoveries, we hypothesize that: **1)** HMGA1 is a key regulator in gut stem cell function, and, **2)** Strategies that activate HMGA1 expression could be harnessed to generate miniguts enriched in stem cells to repair injured or diseased intestines for patients. Using our unique resources and expertise, we now propose studies to test these hypotheses with the following:

Specific Aim 1) To define the role of HMGA1 in human ISC homeostasis in preclinical mouse models and human gut organoids

Specific Aim 2) To develop technology to generate human miniguts enriched in stem cells for gut regeneration by transplantation into a preclinical model of a common intestinal disease (inflammatory bowel disease).

Results from our studies will not only shed light on how ISCs work, but should also reveal new approaches that could be harnessed in the clinics to repair damaged gut tissue to benefit Maryland citizens and others with intestinal diseases.

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Award Amount: $218,500
Disease Target: Parkinson's Disease

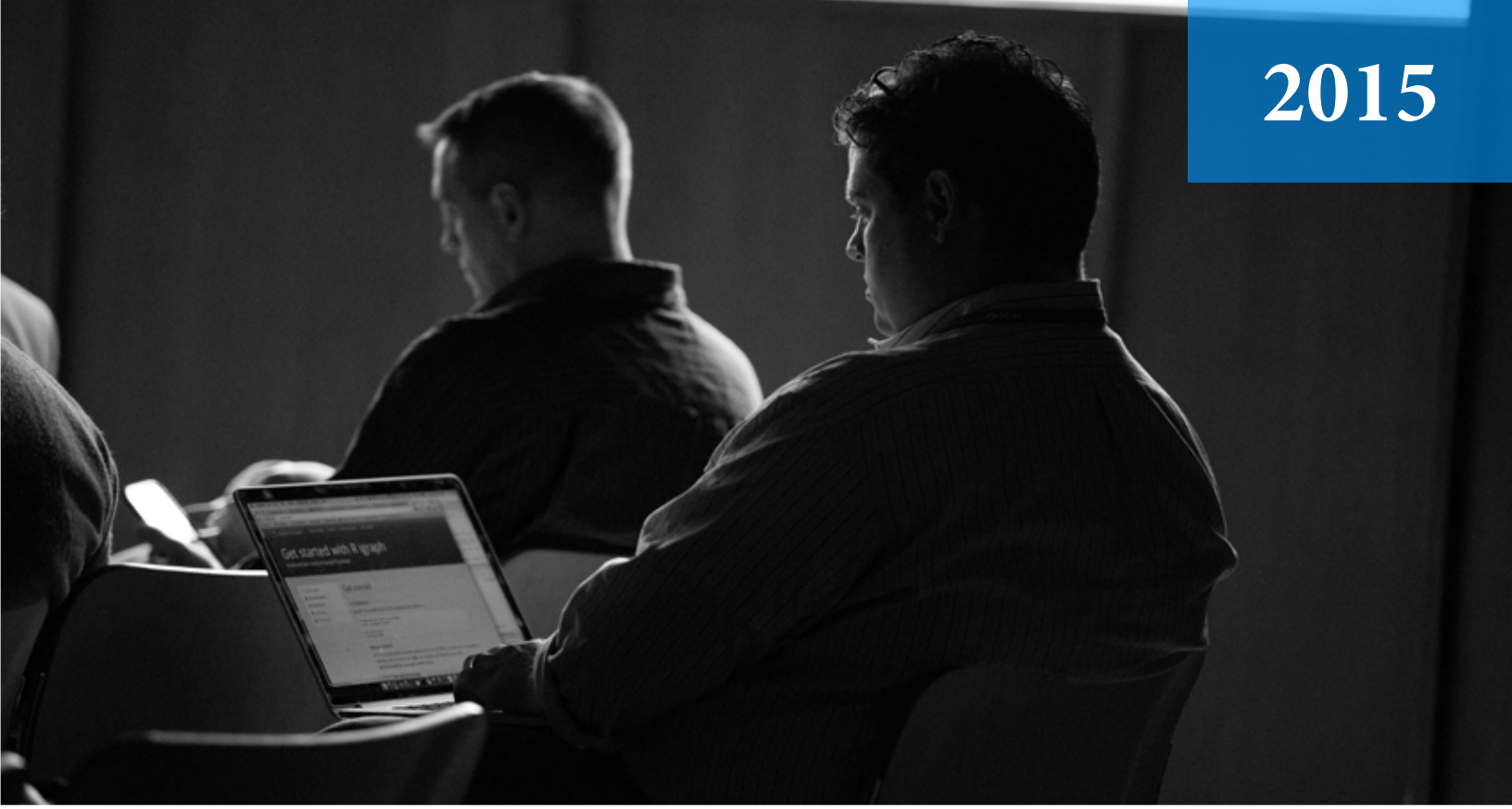
Highly Efficient Conversion of Human iPS Cells to Dopaminergic Neurons by Synthetic Modified mRNAs

Parkinson's disease (PD) is a degenerative disorder of the central nervous system, mainly caused by the death of dopamine-generating neurons in the midbrain (designated as mDA neurons). Human mDA neurons derived from induced pluripotent stem cells (iPSCs) provide a unique cell resource for disease modeling, drug development and cell replacement therapy for PD. Current methods for generating transplantable iPSC-derived mDA neurons are still slow and variable. These methods rely on using multiple growth factors and chemical compounds to indirectly activate essential transcription factors (TFs) (e.g. FOXA2, LMX1A, NURR1) to drive mDA neuron differentiation. Here, we propose to directly activate these essential TFs by delivering synthetic mRNAs to express these TFs, aiming at more rapidly and efficiently differentiating iPSCs into mDA neurons. Our preliminary studies support that such strategy is feasible and highly efficient. We have successfully generated highly pure and functional iPSC-derived mDA neurons by sequentially delivering synthetic mRNAs coding four TFs (ATOH1, FOXA2, LMX1A and NURR1, referred to as "AFLN TFs"). Our novel approach is safe, convenient and reliable, and it does not rely on growth factors, chemical compounds or viruses that are commonly used in other methods. In this project, our goal is to establish an optimal approach for generating highly pure and functional mDA neurons from iPSCs, and further translate this approach into products for generating mDA neurons for PD disease modeling and cell replacement therapy.

In Aim 1, we propose to optimize our AFLN-mRNA-driven strategy by modifying individual AFLN mRNA sequence to enhance TF protein stability and its activity in driving mDA neuron conversion. We will extensively characterize the functions of AFLN-induced mDA neurons in culture and in PD animal models. The goal of this aim is to establish a convenient and reliable approach for rapidly generating highly pure, functional and transplantable mDA neurons from both normal and PD-patient-derived iPSCs. In Aim 2, we propose to identify potential TFs for further improving the AFLN-mRNA-driven strategy. We will globally map the genes that directly activated by AFLN TFs, and further identify TFs that cooperate with AFLN TFs to more potently activate these genes. The goal of this aim is to identify novel TFs that can be incorporated into the AFLN-mRNA-driven strategy to further improve the efficiency of this strategy. Overall, this project is highly innovative and translatable. We will establish a novel approach for rapidly generating highly-pure iPSC-derived mDA neurons, a reliable cell resource for disease modeling and cell replacement therapy for PD. Through collaborating with Molecular Transfer, Inc, a biotech company in Maryland, we will translate our invention (patent pending) into products, such as mRNA-induced mDA neurons and mRNA-driven mDA neuron differentiation kit. Overall, the inventions and methods from this project will facilitate the production of human neurons and other functional cells from iPSCs for research and therapy, and benefit biotechnology in Maryland.

"I urge researchers to make use of the opportunities that are available to them and to do all they can to fulfill the promise that **stem cell research** offers."

-Nancy Reagan-



2015

Post-Doctoral Fellowship Grant Awards:

Xitiz Chamling, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Donald Zack, Ph.D.
Award Amount: $110,000
Disease Target: Multiple Sclerosis (MS) & Demyelinating Diseases

Small Molecules that Promote Differentiation of Myelinogenic Oligodendrocyte Precursor Cells

Multiple Sclerosis (MS) is the most prevalent demyelinating disease and the most common cause of neurological disability in young adults. There are approximately 400,000 MS patients in the US and 2.5 million patients worldwide. In the United States alone, 200 new cases are diagnosed every week. MS is a neurodegeneration in which the immune system attacks and degrades myelin sheaths on axons in the central nervous system (CNS). Myelin is an outgrowth of specialized glial cells: schwann cells in the peripheral nervous system and oligodendrocytes in the CNS. It is an insulating material that coats and protects axons, and enables rapid saltatory conduction. Immune-mediated demyelination in MS interferes with normal nerve conduction and causes apoptosis of the oligodendrocytes, axonal damage, and neuronal cell death. Among the more common presenting complaints of MS patients is optic neuritis, in which demyelination of the optic nerve causes acute, and potentially chronic, visual loss. Currently, all approved therapies for MS focus on modulating the patient's immune response. Although such therapies can be effective in helping to reduce the severity of the relapsing/remitting form of the disease, they do nothing to directly promote remyelination. In fact, there are no clinically tested therapeutic approaches that directly promote remyelination and/or promote neuronal survival . As an approach to developing such therapies, we are interested in helping identify small molecules that can promote remyelination. Recent studies have shown that Oligodendrocyte Precursor Cells (OPCs) can be derived from hiPSCs and the hiPSC-derived OPCs can remyelinate axons in a hypomyelinated mouse model, which suggest that stem cell-based replacement therapy could be a viable option to treat demyelinating diseases. Extensive research on MS and other demyelinating disorders in the past decade has uncovered molecular properties of progenitor cells and their potential to remyelinate damaged axons. Following myelin damage, progenitor cells such as OPCs and neural precursor cells (NPCs) migrate towards the site of injury. The newly arrived OPCs differentiate into new, mature oligodendrocytes capable of remyelinating the demyelinated axons. However, the efficiency of differentiation and remyelination progressively decreases throughout adulthood and it is ultimately insufficient in MS patients to prevent the development of clinical disability. If the damaged myelin is restored, saltatory conduction, axonal integrity and the motor function lost due to demyelination can be regained. Interestingly, even in the MS lesions of the chronic phase of the disease, a significant number of OPCs are present, and if we could use small molecules to target those OPCs to differentiate and become myelinogenic, this could have a great therapeutic value. Additionally, cell-based transplantation approaches could be effective in reducing disability. Therefore, in this project we propose to develop and perform a high-content screen of small molecule libraries to identify lead molecules that promote proliferation and differentiation of myelinogenic OPCs, and thereby help to develop a novel strategy to complement current approaches for MS treatment.

Srinivasa Raju Datla, Ph.D.
University of Maryland, Baltimore
Mentor: Sunjay Kaushal, Ph.D., M.D.
Award Amount: $110,000
Disease Target: Myocardial Infarction & End-Stage Failing Heart

Allogeneic Safety Testing of c-Kit+ Cardiac Stem Cells

Cardiac stem cell (CSC) therapy is developing as an imminent therapeutic option for heart failure (HF). Recent clinical trails using autologous resident CSCs, both cardiosphere-derived stem cells (CDCs) and c-kit positive (c-Kit+ CSCs) cells, have demonstrated the safety and efficacy in adult myocardial infarcted patients.1, 2 Autologous CSCs expansion is an expensive and time-consuming process, and may produce variable results depending on age and co-morbidities. Therefore, its application to the general HF patients is difficult. Allogeneic CSCs obviate these limitations and could be available as a potential off-the shelf product. Our studies identify that adult c-Kit+ CSCs have a decreased growth rate and cardiac regenerative capacity, and they undergo senescence at earlier passages compared to neonatal cells. Understanding immunological responses of c-Kit+ CSCs would help in the promotion of their clinical application. In particular, establishing an allogeneic profile would be helpful, for both adult and neonate HF patients. This study will investigate the in vitro and in vivo immunological responses for c-Kit+ CSCs and evaluate their effects on left ventricular function improvement and cardiac regeneration in allogeneic, syngeneic and xenogeneic settings using rat myocardial infarction models. This information will be critical to design clinical protocols for allogeneic c-Kit+ CSCs. To study this, we hypothesis that c-Kit+ CSCs express low immunogenic profile and produce minimal or no immune response in allogeneic settings. We base this hypothesis on two observations; (1) Human and rat c-Kit+ CSCs expressed intermediate levels of MHC I, but low levels of MHC II and CD86. (2) One way mixed lymphocyte reaction revealed that allogeneic rat c-Kit+ CSCs did not elicit proliferation of alloreactive T-cell, which is comparable to syngeneic c-Kit+CSCs. To further elaborate these finding, we propose to investigate the immunological responses and efficacy of allogeneic rat c-Kit+ CSCs, which would provide the first immune privilege information for the allogeneic c-kit+ CSCs.

Aim 1: Immunological profiling of human and rat c-Kit+ CSCs and in vitro investigation of allogeneic reactivity.

Aim 2: In vivo evaluation of human and rat c-Kit+ CSCs immune responses, cardiac regenerative and functional recovery potentials.

Aim 3: To conduct a CRISPR/Cas9 KO genome screen for rescue of mitophagy in hDA neurons.

Based on our preliminary data we are expected to notice hypoimmuno genic responses to c-kit+ CSCs. However, if we notice hyper-immunogenic response to allogeneic c-Kit+ CSCs, we evaluate cardiac regenerative capacity of allogeneic c-Kit+ CSCs in combination of immunosuppressant therapy.

Jeffrey Ehmsen, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Ahmet Hoke, Ph.D.
Award Amount: $110,000
Disease Target: Muscular dystrophies & ALS

Identifying New Targets to Promote Muscle Regeneration

Muscle atrophy is a loss of muscle mass and corresponding loss of function that occurs in response to diverse stimuli including disuse/immobility, sepsis, hyperthyroidism, diabetes, uremia, starvation, glucocorticoid treatment, cancer, aging, and denervation. Biologically, atrophy reflects the active loss of skeletal muscle contractile proteins and ultimate failure of muscle satellite cell regenerative capacity, leading to loss of strength and functional impairment with substantial impact on quality of life. Denervation is a significant contributor to muscle wasting in trauma, degenerative diseases, and age-associated sarcopenia in humans. Satellite cells, considered resident stem cells of skeletal muscle, possess the unique capacity to proliferate and regenerate in settings of muscle injury, but regenerative capacity becomes depleted during chronic denervation for reasons that remain unclear.

Aim 1: Identify changes that occur in skeletal muscle during chronic denervation.

Aim 2: Identify changes that occur specifically in muscle satellite cells during chronic denervation, and

Aim 3: Investigate novel targets/pathways identified in (1) and (2) for their potential to minimize atrophic changes and/or sustain or enhance muscle satellite cell proliferative and regenerative capacity, using genetic mouse models and human ES cell- (hESC)-derived myotubes and satellite cells.

To achieve these goals, we will first conduct a longitudinal analysis of transcriptional changes occurring in skeletal muscle and specifically in skeletal muscle satellite cells during denervation using a mouse model of denervation injury. We will then use knockdown, overexpression, and small molecule inhibitors/activators of candidate pathways to investigate the conservation of identified pathways in hESC-derived myotubes and satellite cells, and to identify those that may be most promising for clinical application in humans. This proposal describes translational research efforts intending to identify new pathways that may be pharmacologically exploited to delay muscle wasting and/or enhance satellite cell proliferative and regenerative capacity in humans. Our approach will use contemporary genetic tools, sequencing methodology, informatics, and human-derived stem cells to identify and validate transcriptional changes occurring independently in skeletal muscle and muscle satellite cells during acute and chronic denervation. This approach will provide an unbiased characterization of altered and potentially targetable pathways, which will be manipulated in vitro in human stem cell-derived systems to investigate prospects for therapeutic use in humans.

Ileana Lorenzini, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Rita Sattler, Ph.D.
Award Amount: $110,000
Disease Target: ALS & Frontotemporal Dementia

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

Amyotrophic Lateral Sclerosis (ALS) is a neuromuscular disease characterized by the progressive degeneration of cortical, spinal motor neurons and interneurons in affected brain regions. Recent investigations have demonstrated that the most common genetic mutation in sporadic and familial ALS is found in the non-coding region of C9orf72 gene. An abnormal expansion of the hexanucleotide repeat G4C2 is present in this region causing up to 10% of sporadic and up to 40% of the familial ALS as well as up to 10% of familial frontotemporal dementia. Our laboratory used induced pluripotent stem cells (iPSCs) as a valuable research approach to study C9 ALS disease pathogenesis. We discovered specific disease phenotypes in iPSCs differentiated neurons (iPSNs) carrying the C9 mutation such as intranuclear repeat RNA foci, increased susceptibility to cellular stressors and RNA interacting proteins sequestered to the G4C2 repeat. Based on these studies, we hypothesize that the G4C2 repeat expansion can sequester proteins critical for synapse formation and development as well as fast neurotransmission. Increasing evidence links defects in pre and postsynaptic compartments to the etiology of neurodegenerative diseases. We hypothesize that the synaptic dysfunction observed in C9 iPSNs explains the observed increased cellular vulnerability and might also explain cognitive impairment found in C9 patients. Patient-derived IPSNs are a powerful tool to determine disease mechanisms and to evaluate future neurotherapeutics, which is why we propose to use iPSNs carrying the C9 mutation to determine structural and functional deficits in synapses.

Aim 1: **To determine whether patient-derived C9orf72 iPSNs present with aberrant spine morphology when compared to control iPSNs:** This aim will provide a basic understanding on the role of mutant C9 on the structural and biochemical organi zation of the excitatory synapse and consequently the synaptic homeostasis. Although the proposed use of the eGFP-lentiviral construct has provided us with good quality data thus far, if needed, we could replace the standard eGFP with a membrane-targeted GFP to improve on the visualization of individual spine structures.

Aim 2: **Assessment of the molecular mechanisms of synaptic dysfunction in C9orf72 iPSNs: dipeptide repeat proteins (DRPs) versus repeat RNA interacting proteins:** The results of this aim will provide insights into the molecular mechanisms of the observed synaptic dysfunction from Aim 1 and will therefore likely lead to the dentification of novel therapeutic targets for C9orf72 drug development. We do not anticipate any difficulties with the described methods.

Sudhish Sharma, Ph.D.
University of Maryland, Baltimore
Mentor: Sunjay Kaushal, Ph.D.
Award Amount: $110,000
Disease Target: Myocardial Infarction, Cardiomyopathy

Characterization of the Secretome of ckit+ Human Cardiac Stem Cells for Mycardial Infarction

Human cardiac stem cells (hCSCs) provide a promising new therapeutic option following myocardial infarction (MI). Despite the regenerative benefit reported in recent human clinical trials, massive death of transplanted stem cells due to high inflammation in the infarcted myocardium reduces the overall outcome of this therapy. The retention of transplanted hCSCs and the rate of differentiation of transplanted or resident hCSCs into cardiomyocytes are too low to explain the myocardial regeneration. This discrepancy has led to the paracrine hypothesis of stem cell action that indicates communication between newly transplanted stem cells and reparative native cells, essentially jumpstarting the repair process. Thus, it is very important that we understand the paracrine growth factors and cytokines of the total secretome of hCSCs and work towards improving its therapeutic potential. My mentor Dr. Kaushal is one of the few pediatric surgeons in the country that has isolated hCSCs from very young cardiac tissue. Our preliminary studies has shown that hCSCs derived from the biopsies of right atrial appendage of young human subjects are significantly more efficient in myocardial regeneration and functional recovery as compared to hCSCs derived from adult human. This robust nature of yhCSCs does not appear due to better transplantation or retention of cells in the host myocardium but is likely attributable to the secretion of important cytokines and signaling molecules. In evidence of this, our preliminary analysis of total secretome from yhCSCs and ahCSCs identified many proteins in yhCSCs known to reduce cell apoptosis and tissue inflammation, and increase angiogenesis and proliferation while ahCSCs expressed fewer angiogenic and anti-inflammatory proteins and a higher number of pro-apoptotic proteins. In addition, our recent publication supports that the heat shock factor 1(HSF1) is necessary and sufficient to enhance the regenerative functional activity of the secretome from cardiosphere derived cells but this pathway has not been tested to effect the secretome in c-kit+ hCSCs. To directly benefit the older person more at risk of heart disease, it is important to understand and improve the secretome of their ahCSCs. The overall goal of this application is to define the secretome of ahCSCs by specific and sensitive readouts of functional activity. This study will increase the therapeutic potential of ahCSCs by enhancing the angiogenic and proliferative potential of its secretome.

Hideki Uosaki, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Chulan Kwon, Ph.D.
Award Amount: $110,000
Disease Target: Heart

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

Human pluripotent stem cells (PSCs) hold great promise for patient specific disease modeling and drug discovery. Cardiomyocytes (CMs) are the one of most desired cell types for the applications because heart disease remains a leading cause of death worldwide1. Although generating fully mature CMs is essential to study adult-onset diseases with PSC-derived CMs (PSC-CMs), factors and mechanisms regulating CM maturation remain unclear and, consequently, no method is available toobtain mature CMs from PSCs. Therefore, it is crucial to understand the factors and mechanisms mediating CM maturation. Micro RNAs (miRNAs) regulate a large number of target genes with specific biological functions through binding to 3' untranslated region (UTR)2. While cardiac deletion of Dicer1, a key regulator of miRNA biogenesis, suggested an essential role of miRNAs in CM maturation, it remains unknown which miRNAs are involved in the process3,4. During the course of CM maturation, increased contractility is accompanied with decreased CM proliferation. With bioinformatics approaches, I have identified miR-139/6715 and let-7 as potential upstream factors that negatively regulate genes involved in CM contractility and proliferation, respectively. Hence, I propose to test the hypothesis that CM maturation is negatively regulated by miR-139 and miR-6715 and positively regulated by let-7.

Aim 1: To determine if miR-139/6715 negatively regulate CM maturation I will manipulate miR-139/6715 expressions in developing heart and human PSC-CMs and determine their target genes affecting CM maturation. The results will show if and how miR-139/6715 affect CM maturation.

Aim 2: To determine if let-7 positively regulate CM maturation Let-7 miRNA is known as a negative regulator of cell cycle, but it is unknown if it affects CM maturation. I will manipulate let-7 expression levels in postnatal heart and PSC-CMs. The results will show if let-7 regulates not only cell cycle progression but also CM maturation.

The goal of the proposed study is to understand the molecular mechanism by which CM maturation is regulated via miRNAs. To do this, I will utilize mouse hearts and human PSCs and perform loss- and gain-of function experiments. By the end of the project, I anticipate to better understand the process of CM maturation, which may lead us to generate mature CMs from human PSCs for disease modeling and drug discovery of adult heart diseases.

Sooyeon Yoo, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Seth Blackshaw, Ph.D.
Award Amount: $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 how changes in the function and connectivity of these cells leads to obesity. Adipose cells directly signal to the brain through secretion of leptin. Despite high concentration of circulating leptin in obesity, hypothalamic neurons don't respond to leptin and lose their ability to repress feeding. Over-coming this obstacle is a top priority in conquering obesity. Unlike other regions of the hypothalamus, the median eminence lies outside the blood brain barrier, allowing rapid and sensitive respond to dietary-regulated blood-borne signals including leptin. Recently, our group identified the hypothalamic ME as a site of active neurogenesis in postnatal and adult mic e and found that blocked neurogenesis by CT-guided focal X-ray irradiation of the ME significantly reduced the rate of weight gain in high-fat diet (HFD)-fed young adult mice, notwithstanding HFD- induced leptin resistance. This study proposes using transgenic mice to better understand the role of postnatal and adult-generated ME neurons in regulating body weight, and xenografting human embryonic stem cell (hESC)-derived tanycytes to determine whether findings from mice are directly translatable to humans. We expect this study to point the way towards novel therapeutic approaches for treating obesity and type 2 diabetes .

There is now compelling evidence that adult hypothalamic neurogenesis regulates body weight, but the mechanism by which this occurs is poorly understood and still studied by few groups. By characterizing the neuronal subtypes generated from neurogenic tanycytes, and using genetic tools to selectively ablate newborn neurons, I will greatly expand our knowledge of this topic. Although we now know a good deal about how the brain regulates body weight in mice, little of this has yet been directly translatable to humans. By generated tanycyte precursors through directed differentiation in vitro, and using retroviral labeling in combination with xenografting, I will determine whether human tanycytes also function as diet-regulated neural progenitor cells in vivo. These animals may eventually prove useful in testing therapies for human disease. This combination of basic science and translational, hESC-based studies is thus highly innovative.Our group has identified the hypothalamic ME as a site of active neurogenesis . HFD triggers the birth of new ME neurons, and inhibiting HFD-induced cell proliferation in ME with focal irradiation significantly decreases body weight and elevates activity levels. Based on these results, I hypothesize that ME neurons play a critical role in controlling feeding and energy balance, and that regulated production of new ME neurons influences these processes in adult.

Ki-Jun Yun, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Hongjun Song, Ph.D.
Award Amount: $110,000
Disease Target: Schizophrenia, Autism, & Mental Disorders

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

Neuropsychiatric disorders are debilitating conditions that are postulated to have a neurodevelopmental etiology. 15q11.2 CNVs have emerged as prominent risk factors for various neuropsychiatric disorders. Interestingly, 15q11.2 has been consistently found to be strongly associated with schizophrenia (SCZ) in the context of a microdeletion and associated with autism (ASD) when duplicated. In addition, a recent study using various tests of cognitive function further shows that control subjects carrying the 15q11.2 CNVs perform at a level between that of schizophrenia patients and population controls and structural MRI scanning further showed dose-dependent alterations in brain structures in 15q11.2 CNV carriers. However, it is largely unclear how different doses of genes within 15q11.2 region result in different cellular outcomes and contribute to etiologies of SCZ and ASD. To generate appropriate model systems to resolve this question, I established iPSC lines from four individuals carrying 15q11.2 deletion (15q11.2-del) and two individuals carrying 15q11.2 duplication (15q11.2-dup). Initial analysis of iPSC-derived neural progenitor cells with 15q11.2 deletion revealed impairments in adherens junctions and polarity due to WAVE complex destabilization by haploinsufficiency of CYFIP1, one of four genes within 15q11.2. In addition to defects of adherence junctions in the developing mouse brain using in utero CYFIP1 knockdown, neural migration is also affected, suggesting that CYFIP1 may be important for multiple cellular processes in the developing brain. Because conventional differentiation in 2D culture cannot recapitulate organized features of human brain development, such as radial migration of newborn neurons and cortical layer formation, I will use a recently developed 3D cerebral organoid system to examine structural brain development using iPSCs with different copy number of 15q11.2. The specific functional contributions of different doses of 15q11.2 to neurodevelopment and neuropsychiatric disorders in humans are still largely unknown and will be the major focus in this proposal.

Aim 1: To generate cerebral organoids from iPSC lines with 15q11.2-del/dup.

Aim 2: To characterize neural progenitors in cerebral organoids with 15q11.2-del/dup.

Aim 3: To investigate neuronal migration and cortical layer formation in cerebral organoids with 15q11.2 del/dup.

15q11.2 CNV is one of the best characterized genetic risks associated with multiple psychiatric disorders. Investigation of the cellular and developmental roles of 15q11.2 CNV mayprovide molecular insights into the functional roles of altered dose of 15q11.2 as a putative etiological factor in ASD, schizophrenia and other neuropsychiatric disorders.

Yunhua Zhu, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Hongjun Song, Ph.D.
Award Amount: $110,000
Disease Target: Microcephaly, Lissencephaly

Single Cell Transcriptome Analysis of Human iPS-Derived Neural Progenitors

Subtle differences in transcriptomes among phenotypically similar neural progenitor cells (NPCs) may drive them into different fates, and precise understanding of these initial differences are essential for understanding brain development and its dysregulation. Using single-cell transcriptome analysis, it is now possible to identify subtle heterogeneity among NPCs and create a holistic overview of fate determination. We have successfully validated this strategy using mouse NPCs. In this application, I will use single-cell transcriptome analysis to investigate the fate specification of human cortical neurons using induced pluripotent stem cell (iPSC)-derived cortical NPCs. Transcriptomes of individual NPCs at different stages of differentiation will be analyzed using a suite of algorithms that include unsupervised clustering. Gene ontology analysis will be done to identify signature molecular architecture of each cellular state. This study will provide the first characterization of the dynamic process of fate determination in human neural progenitors based on the dynamic progression of gene transcription.

Neural stem cells make fate decisions to generate different types of progenitors and neurons. Precise control over fate choice guarantees the correct number and proportion of different types of neurons and glia, therefore determine the resultant integrity of neural architecture and function. Dysregulation of cell type specification, migration and integration can result in brain disorders such as microcephaly or lissencephaly (Manzini, 2011). For these reasons, understanding of the precise mechanism governing fate determination is a fundamental challenge in stem cell biology and of paramount importance for understanding of disease mechanisms and for generating specific cell types for tissue repair (Gage, 2013).

Fate determination takes place at individual cell level, starting from signaling initiated outside the cell or possibly from internal timing mechanism within the cell, and proceeds with subtle changes in expression architecture before a cell fate can be fully executed (Leone, 2008; Molyneaux, 2007). Currently, detection of fate choices are routinely done by staining of known molecular markers (Leone, 2008; Molyneaux, 2007), which is biased by prior experimental knowledge and may have left out important intermediate stages of differentiation that is minor in population size and/or that may have no distinct marker expression (Treutlein, 2014). I propose that the process of fate determination can be best visualized using unbiased methods such as single cell whole genome transcriptome analysis.

One excellent model system to study fate determination is the development of cortical projection neurons. It is well established that radial glia (RGs) in the ventricular zone are neural stem/progenitor cells (NPCs). At population level, NPCs give rise to neuronal subtypes in deep layers first (expressing Brn1 then Ctip2), followed by neurons in superficial layers (expressing Satb2) in a sequential, inside out manner (Leone, 2008; Manzini, 2011). Interestingly, sequential generation of different subtypes of neurons of different layers can be recaptured during in vitro differentiation from ES/iPSC derived cortical NPCs (Shi, 2012; Wen, 2014), indicating there may be an internal timing mechanism within NPCs that control the sequential fate determination. iPSC induced NPCs (90% expressing Pax6+, FoxG1+) generate deep layer neurons (layer IV/V, Tbr1+, Ctip2+) before generating neurons of superficial layers (Brn2+, Satb2+). Consistently, transplantation experiments show that early NPCs generating neurons of deep layers, when transplanted to brain of a later stage, can switch their fate to generate neurons of superficial layers; whereas the reverse did not happen (Leone, 2008; Pakic, 2009), suggesting that early NPCs are more flexible in their fate choices than late NPCs. However, the cellular and molecular mechanisms that differentiate early vs late NPCs is not clear. It is possible that early and late cortical NPCs, though with similar morphology, are at different cellular states with different architecture in transcriptome, alternatively, early NPCs as a heterogeneous population may contain certain early lineage restricted precursors that are exhausted in the late NPC population. Analysis of transcriptome of individual NPCs at different stages of differentiation will enable us to distinguish these different possibilities and provide a high resolution and complete overview of different cellular states along neuronal fate determination.To this end, we have recently established single-cell RNA sequencing and developed an analytical pipeline in the lab to visualize whole-genome transcriptome of Nestin positive cells in adult hippocampus neurogenesis. Using this methodology, we have uncovered multiple, previously unrecognized, cellular states within Nestin positive cells in mouse. We were able to identify up- and down-regulation of gene sets related to different cellular processes along the continuum of neuronal differentiation and established a high resolution road map for stem cell commitment to neuronal lineage. In this current proposal, I will leverage this single-cell transcriptome analysis to investigate the fate determination in human iPSC derived cortical NPCs, to understand how cortical NPC generate precursors of different neuronal subtypes. To do that, I will choose multiple time points and sequence individual NPCs during the process of cortical neurogenesis for analysis.

2015

Completed Awarded Research:

{Calendar Year 2015}

Valina Dawson, Ph.D.
Johns Hopkins University
2009 Investigator Initiated Award Budget: $1,725,000
Disease Target: Multiple

Neuroprotective Pathways in iPS Derived Human Neuronal Cultures

Stroke is the third leading cause of death and disability in the USA. Extensive studies have been conducted in small anima l models, but therapeutics developed from these models have not translated into clinical improvement in human patients. This raised a concern that perhaps key aspects of ischemic and neurotoxic injury in human neurons are unique. To date this question has not been answerable. Unfortunately existing protocols to study mechanisms of neurotoxicity in human neurons are limited to either the expression of excitatory or inhibitory neuronal populations. This is a major short coming because neuronal nitric oxide synthase (nNOS) neurons which are inhibitory neurons are inquired for cell death both in vitro and in vivo. Thus both populations, excitatory or inhibitory, are required to properly explore neurotoxic mechanisms and define protective strategies in human cortical cultures. Therefore we developed a new methodology to differentiate human embryonic stem cells or induced pluripotent stem cells into all classes of cortical excitatory and inhibitory neurons in a balanced manner reflecting the mature human cerebral cortex. Our approach is an advance over prior protocols and important to investigators interested in neurologic disorders and diseases of the human cortex and the development of new therapeutic strategies. Importantly, using this newly develop human neuronal culture paradigm, we find that Parthanatos occurs in human cortical neuronal cultures in a manner similar to that observed in rodent model systems. We believe this is critically important as it indicates for the first time that findings in other mammalian systems are relevant to the human condition. Further we show that PARP inhibitors that are currently in clinical trials for oncologic indications, attenuate neurotoxicity in human cortical cultures.

These data indicate that PARP inhibitors should be advanced into clinical trial for the treatment of stroke and other neurologic conditions. Additionally, we have defined neuroprotective preconditioning in mature cortical cultures. We have analyzed our deep sequencing data and found that expression patterns are similar for NMDA and OGD precondition ing but that there are expression patterns unique to OGD that are not shared by NMDA preconditioning. This indicates that there may be multiple signaling pathways and thus opportunities to initiate neuroprotection. Recently we have discovered an epigenetic role for PARPl and parsylation in preconditioning and neural plasticity. In analyzing the deep sequencing data in relation to PARPl we believe that we have discovered a master regulator. This is important as it could provide a critical therapeutic target to elicit neuroprotection. Translational Potential of Project: We have developed, characterized and standardized human cortical cultures for the study of neurotoxicity. We have defined neurotoxicity platforms for the study of stroke, the third leading cause of death and disability in the USA. This technology can be used for discovery and validation of agents targeted towards treating neurologic disease. We have found neuroprotective pathways in human neuronal cultures that can be developed as therapeutic targets. The identification of Parthanatos in human cortical neurotoxicity is an important finding to encourage pharmaceutical companies to translate these findings and advance PARP inhibitors into clinical trials for stroke. Thus the goals of this project directly impact the state of Maryland biotechnology that is interested in experimental investigation in neurologic disease, drug screening and ultimately clinical purposes.

Curt Civin, Ph.D.
University of Maryland, Baltimore
2010 Investigator Initiated Award Budget: $1,149,989
Disease Target: Cancer

Hematopoietic Stem Cell-Enriched MicroRNAs in Human Stem Cell Differentiation and Self-Renewal

My laboratory seeks to better understand the pathophysiology of normal blood-forming hematopoietic stem progenitor cells (HSPCs) and leukemias. Propelled first by my lab's discovery that a number of the tiny snippets of RNA of the class called microRNAs are selectively expressed in human HSPCs, we and others have shown that certain microRNAs can be powerful post-transcriptional regulators of HSPC and leukemia biology. The overall goal of this MSCRF project was to further understand the effects of microRNAs in early hematopoiesis. We hoped that this deeper understanding might lead to specific new approaches to expand the quantities of HSPCs for clinical transplantation and of more mature blood cells for transfusion therapies, as well as provide new molecular targets for leukemia treatment. In this MSCRF project, we identified and evaluated multiple microRNAs and related molecules that affect HSPC and LSC functions and identified some of novel target molecules that contribute to the mechanisms of these effects. Both of these sets of discoveries have direct translational implications. In an example of the translational power of one set of our discoveries, we found that several microRNAs are expressed at only low/absent levels in many leukemias, as compared to normal HSPCs or more mature hematopoietic cell types. Since (re)expressing these down regulated microRNAs in leukemias reduced cell proliferation and increased apoptosis, we considered the potential of these and other leukemia suppressive microRNAs for antileukemic therapies. However, while systemic delivery of microRNAs has promise for the future, there are still no clearly effective means to deliver a variety of microRNAs to cancer cells at all sites in the human body. As an alternative, we investigated the idea of discovering small molecule drugs that alter the levels of specific microRNAs in human cells, which would open a novel strategy for treatment of leukemias (and other cancers). Via high-throughput screening of clinical drug libraries, we identified a new set of drugs that selectively upregulate miR-34 and/or other leukemia suppressive micro-RNAs.

Among validated lead candidate drugs from our high-throughput drug screens, the Artemisinin antimalarials are particularly exciting,because of their known broad preclinical antineoplastic efficacy with low clinical toxicity. Our ongoing studies show that Artemisinins have significant potential to be repurposed for treatment of leukemias. A second new translational project started from our discovery in this MSCRF project that RAB GTPase14 is targeted by both members of the miR-144-451 micro RNA cluster, which we showed to be selectively expressed and functionally important during human erythropoiesis. Expression of RAB GTPase14 protein decreases during erythroid differentiation, and lentiviral shRNA-mediated RAB14 GTPase knockdown increased the frequency and total numbers of erythroid cells and decreased expression of the ET02 erythroid transcriptional repressor, which in turn controls expression of several globin genes. Thus, RAB GTPase14 is a novel endogenous physiologic inhibitor of normal human erythropoiesis (17). Since RAB GTPase7b and RAB GTPase27b have been reported to be involved in megakaryocytic differentiation, and since we had found separately in this MSCRF project that RAB GTPase5C overexpression inhibited growth of human leukemia cells (18), we evaluated the erythropoietic effects of RAB GTPase5C and our ongoing results suggest that RAB GTPase5C is a novel positive erythropoietic regulator. Therefore, we have launched a new project to investigate the cellular and molecular mechanisms by which RAB GTPase14 and RAB GTPase5 modulate normal human hematopoiesis. By determining how RAB GTPases regulate hematopoiesis, we hope to identify novel therapeutic targets for ex-vivo manipulation of HSPCs and erythroid progeny to generate the numbers of cells needed for clinical transplantation and/or transfusion products.

Feyruz Rassool, Ph.D.
University of Maryland, Baltimore
2011 Investigator Award Budget: $662,998.30
Disease Target: Regenerative Treatment

Remodeling the DNA Damage Response in Induced Pluripotent Stem Cells

Generating human induced pluripotent stem cells (hiPSCs) from adult cells represents one of the most exciting developments in regenerative medicine. However, potential clinical applications of hiPSCs are severely hampered by low efficiency of production and sub-optimal genomic integrity. One of the key pathways for maintenance of genomic integrity is correct repair of DNA double strand breaks (DSBs) that can occur through exposure to endogenous or exogenous DNA damaging agents. Incorrect or error-prone repair can lead to mutations and genomic instability. Recent studies have demonstrated that stringent and error-free DSB repair properties not only maintain genomic integrity of hiPSCs, but also improve the efficiency of generating hiPSCs. The goal of our studies was to determine whether the methods used to derive h/PSCs influence the DNA damage and repair response (DDRR) and therefore the potential for genomic instability of these cells. We also wished to elucidate the factors that regulate the DSB repair response, in particular the error-prone non-homologous end-joining (NHEJ) pathway in h/PSCs compared with that of human embryonic stem cells (hESCs). Achieving these goals would ultimately improve reprogramming efficiency while at the same time maintaining pristine genomic integrity of these cells for use in regenerative medicine.

In collaboration with the Zambidis laboratory (JHU, co-investigators), we first examined expression profiles for DNA repair genes in mRNA from parental cells (cord blood [CB] and adult fibroblasts) from which IPSCs are derived, hiPSCs and hESCs, using microarray analysis. We found that expression profiles for a subset of key DNA repair genes (including Ku70/80 and PARP1), CB appeared to be more similar to hESCs. In contrast, expression profiles in adult fibroblasts for this same set of genes was quite distinct from hESCs. Reprograming of parental cells irrespective of their origin led to a DNA repair expression profile that was similar to those of hESCs.

Discovery 1: Our work suggests that reprograming of pluripotent cells from CB would likely not require extensive reprograming, compared with those of more differentiated cells such as fibroblasts. We next examined the DDRR in a variety of hiPSCs, including those derived using viral vs non-viral methods and compared results with those of hESCs.

Discovery 2: Both virally and non-virally derived hiPSCs demonstrate increased repair of DNA damage and increased apoptosis in response to ionizing radiation (2Gy) and exhibit similar responses to those of hECSs.

Finally, we focused in depth on ascertaining whether the methods involved in increasing the efficiency of reprograming of hiPSCs were also involved in the DDRR and maintenance of genomic integrity. Studies from the Zambidis lab showed that hematopoietic growth factors (GF) + bone marrow stromal cell (MSC)-priming accelerated reprogramming of CB progenitors (CB.iPSCs +MSC), compared with those derived without MSC priming CB.iPSCs (-MSC). In the collaborative publication with Dr. Zambidis, we demonstrated that the vascular progenitors derived from CB.iPSC(+MSC) possessed greater ability to withstand DSB damage inflicted by ionizing radiation.

Discovery 3: CB.iPSCs (+MSC) have levels of error-prone NHEJ repair that is very similar to that of hESCs. In contrast, CB.iPSCs (-MSC) had a significantly higher number of repair errors. One of the key features of MSC-activated CB.iPS+MSC is that they possess hESC-like MYC regulated gene expression profile. MYC is a master transcription factor in regulation of genes in hESCs. Therefore, we questioned whether C-MYC contributed to enhanced integrity of repair in CB.iPSCs (+MSC). We found that inhibition of MYC leads to an increase in error-prone NHEJ repair. These results imply that a MYC gene expression signature is linked to efficacious NHEJ DSB repair in pluripotent cells.

Discovery 4: Our results also indicate that expression of a MYC gene expression profile in hiPSCs could not only be an important indicator of overall efficiency of reprogramming, but also overall DDRR and in particular, repair of DSBs. This work is being prepared for publication.

In conclusion, our studies show that mechanisms for generating hiPSCs with high efficiency are linked to pathways that regulate genomic integrity. Further elucidation of the role of MYC in maintenance of genomic integrity, regulating efficacious repair of DNA damage in pluripotent cells is required.

Gerald Brandacher, Ph.D.
Johns Hopkins University
2012 Exploratory Award Budget: $229,905
Disease Target: Regnerative Treatment

Induced Pluripotent Stem Cell (iPS) Derived Schwann Cells to Enhance Functional Recovery Following Nerve Injury and Limb Allotransplantation

Hand and upper extremity transplantation represents a bona fide treatment option for reviving formand function of an amputated hand or arm. Optimal functional recovery, however, remains the major challenge for this innovative treatment option. In this project we developed a novel cell based therapy capitalizing on human induced pluripotent stem cell {iPSC) derived Schwann Cells (SC) to enhance nerve regeneration and functional outcome in a rodent model of chronic denervation and limb allotransplantation.

Aim 1: To determine the effect of hydrogel-based sustained delivery of neurotrophic factors in a rat chronic denervation model of peripheral nerve injury: A fibrin-based sustained delivery method was first optimized in vitro, and then applied in vivo at the nerve repair site to deliver specific growth factors to regenerating nerves in our chronic denervation model. Thereby either Glial Cell-line Derived Neutrophic Factor {GDNF), chondroitinase, GDNF+chondroitinase, or fibrin gel only was applied to the distal stump of repaired nerves. Our findings demonstrate that early measures of nerve regeneration (e.g. histomorphometry and retrograde labeling) after delayed nerve repair are best improved by targeting axonal regrowth (with GDNF) and scar tissue breakdown (with chondroitinase).

Aim 2: To determine the survival and neuroregenerative impact of iPSC-SCs and SC overexpressing neurotrophic factors delivered to the end-to-end nerve repair site following chronic denervation. We optimized our protocol to derive neuronal and SC lineages from human embryonic cells and thus to generate iPSC lines plus the differentiation of those iPSC to SC precursors and SC like cells. For SC differentiation, we iso lated CD49d+ cells that exhibit high level of SC marker gene expression {>90%). Utilizing the chronic denervation model we investigated the impact of iPSC-SCs delivered to the nerve repair site in comparison to a control group (no treatment, nerve repair only). In addition, a group using muscle derived stem cells {MDSC) was also investigated as a novel strategy. Initial results demonstrated improved functional recovery assessed with the Noldus Catwalk gait analysis system in iPSC-SC and MDSC treated groups in comparison to the control group without stem cell therapy.

Late time point histomorphometry results further demonstrated a trend towards improved axonal regeneration and fiber maturation favoring the iPSC-SC treated group compared to the MDSC group.

Aim 3: To compare direct release to cell- mediated release of growth factors for improvement of functional nerve recovery in the setting of limb transplantation. We optimized a translational animal model for limb transplantation to investigate the neuroregenerative effects of iPSC-SCs. A rat orthotopic hindlimb transplant was performed in combination with chronic denervation of the sciatic nerve. At the time of nerve repair either iPSC-SCs or no cells were administered to the repair site. iPSC-SCs showed a comparable trend towards improved nerve regeneration after hind limb transplantation as was observed in the chronic denervation model, however, final data analysis of experimental groups in Aim 3 is currently still ongoing.

Key Achievements:

- We optimized animal models for translational studies to investigate neuroregenerative modalities in chronic denervation and limb transplantation.
- We developed and optimized a protocol to derive neuronal and SC lineages from human embryonic cells and thus to generate iPSC lines plus their differentiation to SC precursors and SC like cells.
- We demonstrated significantly enhanced nerve regeneration using sustained delivery of neurotrophic factors (GDNF) and scar tissue breakdown {chondroitinase).
- In order to further protect the nerve repair site, we devised a novel nanofiber nerve wrap and secured funding from American Society for the Surgery of Hand, and patent was filed with USPTO.
- Relevant manuscripts, intellectual property, and further grants for pre-clinical large animal trials are being compiled with potential downstream impact for the economy of the State of Maryland.

We thank the MSCRF for the generous support to make this project possible.

Gabriel Ghiaur, Ph.D.
Johns Hopkins University
Mentor: Richard Jones, Ph.D.
2012 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Schizophrenia

Retinoic Acid (RA) Controls Self Renewal & Differentiation of Human Hematopoietic Stem Cells (HSCs)

Advances in regenerative medicine and stem cell therapies are hindered by the scarcity or stem cell and our inability to maintain or expand these cells ex vivo. This project proposed to understand in modulation of retinoic acid (the active compound of vitamin A) could lead to better culture conditions for ex vivo manipulation of human hematopoietic stem cells (HSC). In addition, we strived to understand how the bone marrow microenvironment controls HSCs homeostasis. Better understanding of such mechanism could lead to new tools for HSC expansion.

We have sorted and anyalzed the HSC compartment (CD34+CD38-) and the hematopoietic progenitor cell (HPC) compartment CD34+CD38+) from the bone marrow of healthy volunteers and compared their transcription profile to identify that RA pathway was differentially activated between the HSC and HPC.

Using a RA inhibitor, we have expanded human HSCs during culture conditions showing proof of concept that blocking RA pathway could improve current protocols for human HSC expansion.

In addition, we have identified that inhibition of RA signaling appears to be a physiological way by which HSC are maintained in vivo. Since bone marrow microenvironment metabolize RA via CYP26 activity. This could explain for instance how some physiological stressors can change HSC behavior and adapt hematopoiesis to increased demand.

Results generated by these studies have been well received by the scientific community and have been published in PNAS, 2013.

Miroslaw Janowski, Ph.D.
Johns Hopkins University
2012 Exploratory Award Budget: $230,000
Disease Target: Stroke

Magnet-Navigated Targeting of Myelin Producing Cells to the Stroke Via Intraventricular Route in a Large Animal Model

In the first year of the grant initial experiments on pigs were performed using intraventricular delivery of cells. Real-time MRI guidance revealed that this route of delivery is feasible in pigs, and iron oxide labeled cells can be visualized on a clinical scanner. Initially we evaluated the initial distribution of transplanted cells in pigs as a baseline for magnetic neuronavigation. We have shown that cells transplanted into frontal horn of lateral ventricle of pig are immadiately distributed within a time-frame of less than one minute. The cells are flowing toward the occipital horn and they travel via 3rd and 4th ventricle into the basal cisterns of the posterior fossa. Thus after one minute the cells are present in the frontal and occipital horn of ipislateral hemisphere and in the basal cisterns of posterior fossa such as cisterna magna and the cistern of C-P angle. Based on that baseline we have performed initial experiments with magnetic navigation of glial progenitors, as it was planned in the grant proposal. The magnet was located in the temporal lobe and the cells were injected slowly into the lateral ventricle. After that the animal was kept for an hour in that position to allow the transplanted cells to adhere tightly to the ventricular wall and anchorage permanently. Then animal was moved to MR scanner and imaged. Unfortunately, we have not observed the predilection of transplanted cells to the frontal horn of lateral ventricle what we expected. In contrast the cells mostly disapperaed from ventricular system, thus the magnetic navigation was not able to provide stable maintenance of transplanted cells in the desired place - in this case in the frontal horn of lateral ventricle. Then we further invastigated the reason for unsuccessful procedure. Since preliminary experiments which showed that this magnetic navigation could be feasible was performed on cord blood derived neural stem cells (CB-NSC), and in the true grant experiments we used glial restricted precursors (GRPs) we perfmormed the detailed comparison of these two populations of cells. We have found that GRPs are much smaller than CB-NSC and by this way they are incorpoating much less iron to the cytoplasm. Then we compared both types of cells in vitro in the magnet-pulling assay and we found that the range of magnetic navigation of very small GRPs is much lower than quite large CB-NSC. Thus we have shown that the effciency of magnetic navigation depends on the cells size and the amount of incorporated iron, and such navigation is potentially feasible for large cells, but is rather difficult for very small cells. In other words for magnetic navigation of small cells there is necessary much stronger magnetic field and it is not feasible to achieve using permanent magnet - the approach we had until now. Therefore we have started collaboration with the company which produces electromagnets and we are working on the appropriate design of electromagnet, which will be enough strong and which shape will fit the needs of magnetic navigation within the ventricular system of both pig and human brain. In the last year we investigated possibility for navigating cells using gradients incorporated into the MR scanners, which would be highly advantageous to have capability both cell visuzalization and navigation as a one-stop-shop during the same procedure performed under MR scanner. We were able to visualize cells during sedimentation and provided some preliminary data that this method mightb be feasible, however it wil need additional funding to get it into realm.

Minoru Ko, Ph.D.
Elixirgen, LLC
2012 Investigator Initiated Award Budget: $690,000
Disease Target: Regnerative Treatment

Generating Human Induced Pluripotent Stem Cells with Less Cancer-Risk

The current paradigm in the field of regenerative medicine is to make induced pluripotent stem cells (iPSCs) from patients' fibroblast cells, differentiate them into desired cell types (such as dopaminergic neurons for Parkinson's disease), and transplant them back to the patient. One of the major concerns of this therapy is the risk that cells derived from iPSCs after transplantation will become malignant and cause more harm than good to patients. For example, it has been shown that the genome integrity of human iPSCs is frequently compromised with mutations, genome alterations, and karyotype abnormalities. It has also been shown that there are hotspots of aberrant epigenetic reprogramming - particularly in the genomic regions near the centromere and telomeres. We haverecently found a few new reprogramming factors that are expressed in a preimplantation embryo-specific manner. Our goal is to test whether these factors can enhance the quality of human iPSCs. We have tested and found that, unlike in mouse iPSCs, the efficiency of generating human iPSCs from human BJ fibroblast cells and keratinocytes is reduced when a preimplantation specific factor is included as one of the reprogramming factors. Following this unexpected finding, we hypothesized that a preimplantation-specific gene can increase the quality of iPSCs by eliminating aberrantly reprogrammed iPSCs. To test this hypothesis, we have examined the epigenetic status of human iPSCs generated with or without a preimplantation-specific gene and found promising results that suggest that the preimplantation-specific factor can improve the quality of human iPSCs. These findings prompted us to further examine whether the same preimplantation-specific gene can function in human fibroblast cells. We have found that euploid cells increased among cultured aneuploid cells after exposure to the preimplantation-specific factor. For example, we have found that within weeks after the application of the preimplantation-specific factor to cells derived from people with Trisomy 21 (Down syndrome), fluorescent in situ hybridization with a chromosome 21-specific probe detected the emergence of up to 24% of cells with only two rather than three copies. Similar observations were obtained for cells with trisomy 18 (Edwards syndrome). We believe that these results open the possibility of improving the genome stability and karyotype of not only human iPS cells, but also human non-immortalized fibroblast cells with trisomy and other chromosome abnormalities.

Jing Fan, Ph.D.
Johns Hopkins University
Mentor: Valina Dawson, Ph.D.
2013 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Stroke

PARP-1 and Histone1 Interplay and Regulate Stem Cell Differentiation after Stroke

The following studies were carried to answer the questions of whether PARP1 interacts with Histone H1.2 to regulate the survival of human embryonic stem cell-derived cortical neurons after NMDA and OGD challenge, and whether Iduna regulates survival of human neuron after NMDA and OGD through degradation of PARsylated H1.2. First, human cortical neurons have been successfully and consistently differentiated from human ES and iPS cell lines, with neurons represent more than 90% of the cultured population and exhibits different cortical layer markers at 1- 2 months post-differentiation. These human cortical neurons at two-month of age are challenged with toxic NMDA and OGD stimuli, with several neuronal death pathway inhibitors including PARP-1 inhibitor DPQ. DPQ, but not other inhibitors, fully protects human cortical neuron from both 30 minutes of 500 micro-molar NMDA and 2 hours of OGD-induced death. Lentiviruses carrying CRISPR/Cas9 sgRNAs targeting PARP-1 are very protective against NMDA/OGD-induced death. Four other PARP-1 inhibitors on clinical trial for cancer therapy abolished PAR polymer formation and fully protect human cortical neurons from NMDA/OGD insults. Lentiviruses carrying shRNAs and CRISPR/Cas9 sgRNAs targeting histone H1.2 have also been made, and are able to knock down/out H1.2 in human cortical neurons and greatly protect against NMDA or OGD-induced neuronal death. Furthermore, H1.2 translocation from nucleus to cytoplasm, and co-localize with PAR polymer, have been observed in human cortical neurons after toxic NMDA/OGD stimulation, which can be reduced when overexpressing wild-type Iduna, but exacerbated when overexpressing an Iduna mutant lacking E3 ligation function. In addition, H1.2 and PAR polymer translocation from nucleus to cytoplasm can be largely reduced by H1.2 shRNAs or sgRNAs in human cortical neurons after NMDA/OGD. These data suggest that PARP-1, H1.2 and Iduna together play critical role in regulating human cortical neuron survival after stroke. Small moleculars targeting PARP-1/H1.2/Iduna pathway can be validated in the human neuron stroke model, and providing drug candidates to help stroke patient recovery.

Jeffery Huo, Ph.D.
Johns Hopkins University
Mentor: Elias Zambidis, Ph.D.
2013 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Hematologic Disorders, Anemias

The Role of Somatic Memory in Determining Efficient Hematopoietic Differentiation of hiPSC

With the support of the MSCRF for this post-doctoral fellowship award, we have demonstrated and are submitting for publication the findings that more complete erasure of hematopoietic donor cell somatic memory during reprogramming to induced pluripotent stem cells (iPSCs) leads to enhanced differentiation potency to hematopoietic (Aim 1) and non-hematopoietic (Aim 2) lineages. These key findings significantly advance the potency, effectiveness,and potential of patient-derived iPSC for translational use. We began by systematically demonstrating that Zambidis laboratory derived myeloid iPSC, with reprogramming optimized through bone marrow stromal priming (sp-myeloid iPSe),have less retention of donor cell-specific somatic memory transcripts than other analyzed classes of iPSC. This is consistent with our second discovery that the transcripts which comprise retained somatic memory are widely present across all tissues, not just blood, and thus perhaps more representative of retention of a general differentiated cell state and less complete achievement of pluripotency. In parallel work, we demonstrated that this same less complete achievement of pluripotency is associated with a greater degree of cancer-like epigenetic derangements. In contrast, more completely reprogrammed Zambidis sp-myeloid iPSC have fewer cancer-like epigenetic derangements. As cancer-like epigenetic derangements acquired during reprogramming to pluripotency are potentially a major safety risk for future translational application, these findings further support the importance and clinical relevance of reprogramming methods which lead to more complete reprogramming. We then showed that these more completely reprogrammed stromal-primed myeloid iPSC, unhindered by retention of somatic memory,are more capable of stable reprograming to a higher state of pluripotency, known as the “naïve” state. In collaboration with other MSCRF-supported investigators in our laboratory, analysis of microarray data demonstrated that sp-myeloid iPSC acquired constellations of gene expression and methylation changes consistent with conversion to the naïve state of pluripotency. Our laboratory further showed that whereas other iPSC lines with less complete erasure of somatic memory were unable to remain in the higher-pluripotency naïve state, sp-myeloid iPSC with more complete erasure of somatic memory stably maintained the higher pluripotency naïve state. Others in the laboratory subsequently demonstrated that these human naïve-state iPSC could differentiate to hematopoietic and non-hematopoietic lineages with even greater efficiency than the already enhanced differentiation potency exhibited by pre-conversion sp-myeloid iPSC. Together, this work clearly demonstrates that patient-derived iPSC generation methods which lead to more complete erasure of somatic memory, and greater fidelity of reprogramming, are crucial to the derivation of more potent, safer SC for translational use.

Anjali Nandal, Ph.D.
University of Maryland, College Park
Mentor: Bhanu Prakash Telegu
2013 Post-Doctoral Award Budget: $110,000
Disease Target: Diabetes

Induced Pluripotent Stem Cell Derived, Immunoisolated β-cell Transplantation for Diabetes Therapy

About 346 million people are affected by Diabetes Mellitus (DM) world-wide and about 4.6 million die due to the resulting complications every year. For DM patients, transplantation of pancreatic islets or β-cells offers an alternative treatment to daily administration of insulin which is beset with several side-effects. However, the β-cells obtained from allogenic donors are limited in supply, are expensive, and often require immune-suppression. Therefore, there is a compelling need for a renewable and reliable source of mature, glucose-responsive β-cells, capable of producing functional levels of insulin. The ability to create iPSC has a huge potential for cellular diabetes therapy as these cells provide a renewable source of cells for differentiation into the lineage of insulin producing β-cells. Therefore, we have generated iPSC using primary human pancreatic cells. The rationale being that iPSC derived from pancreatic cells harbor residual DNA methylation signatures characteristic of their somatic cells of origin and this, epigenetic memory favors their differentiation along the lineages related to the donor cell. The pancreatic cells were reprogrammed using Yamanaka reprogramming factors delivered by sendai virus, which led to the derivation of integration-free iPSC by 25-40 days post-transduction. All iPSC clones are positive for various pluripotency factors and markers like alkaline phosphatase, OCT4, SOX2, NANOG, SSEA-4, TRA-1-60, TRA-1-81 as demonstrated by immunocytochemistry, FACS or RT-PCR analysis. The endodermal cells can subsequently be directed to differentiate to the pancreatic endodermal and β-cell-like lineage using lentiviral vectors encoding the vital pancreatic transcription factors. The cells are currently being modified using CRISPRs targeting insulin antagonists. For this purpose, the CRISPR guides have been cloned, and confirmed to work efficiently in cancer cell lines. The genetically modified ce lls will be assayed for glucose stimulated insulin and/or C-peptide release and then will be transplanted into a diabetic animal model for final maturation and insulin production. These experiments, if successful, would provide a reliable ce ll source for generation of robust human β-cell-like cells, which could be used for diabetes therapy.

Seulki Lee, Ph.D.
Johns Hopkins University
2013 Exploratory Award Budget: $227,536
Disease Target: Cardiac Regeneration

Design of Highly Fluorinated Stem Cells for 19F MR Imaging in Cardiac Repair

Stem cell therapy for cardiac remodeling has shown promising progress towards clinical application; but regardless of its application, stem cell-based clinical trials require effective in vivo stem cell-tracking to follow the distribution and migration of transplanted cells in vivo. Stem cell tracking is vital to monitor therapeutic efficacy, verify safety and optimize dosage of cells. Most contrast agents and detectors are limited in use because they cannot meet the sensitivity to track small numbers of cells throughout the entire body. Recently, one class of magnetic resonance imaging (MRI), 19F MRI, has generated great interest for its application as a hot-spot cell tracking technique. As an alternative to conventional 1H MRI, 19F MRI offers a superior signal-to-noise ratio with no background signals. The inherently low signals of 19F-labeled cells hamper its clinical translation. The goal of this 2-year MSCRF project was to develop an innovative approach to prepare highly fluorinated stem cells with an enhanced detection limit in vivo compared to conventional 19F-labeling techniques.

The approaches were:

- Develop and optimize polymeric nanoparticles consisting of 19F-Active molecules (19F-Nano) that could permeate human mesenchymal stem cells (hMSCs) without inducing toxicity or affecting MSC bioactivity.

- Draft a 19F hMSC-labeling protocol for in vitro and in vivo imaging applications and analyze the in vivo detection threshold of 19F-Nano-labeled hMSCs.

- image and quantify transplanted hMSCs by 19F/1H MRI during cardiac repair During the first year of the MSCRF program, the PI‘s group developed a library of over ten 19F-Nano for in vitro and in vivo preclinical imaging studies (Aim 1) and drafted an hMSC 19F- Nano-labeling procedure that could be completed within 4 hours for in vivo 19F/1H dual MR imaging (Aim 2).

Optimized nanoformulations were chosen based on their safety and uptake into MSCs. In the second year of the MSCRF program, the detection thresholds of 19F-Nano and 19F-Nano-labeled hMSCs were examined (Aim 2), optimized labeling formulations were investigated for their effect on hMSC biological function, including stem cell differentiation (Aim 2), and the in vivo potential of 19F-Nano-labeled hMSC tracking was confirmed in mice (Aim 3). Based on our extensive MR imaging studies in Year 2, we found that the detection signal of 19F-Nano-labeled hMSCs was too low for in vivo cellular tracking. This is an unfortunate drawback of 19F-imaging, as we identified in the “Pitfalls” section of our research proposal.

Therefore, we developed an alternative approach to label hMSCs with 19F-Nano via biorthogonal copper-free click chemistry to meet the detection threshold for in vivo ce llular tracking. Targetable chemical receptors were artificially induced on the surface of hMSCs and complementary targeting groups were added to the 19F-Nano. Using molecular techniques, we confirmed the stability, safety, and binding efficacy of these artificial chemical receptors on hSCs. Next, were-optimized the 19F-Nano formulations labeled with a specific binding group and confirmed a strong 19F MR signal that was proportional to the 19F concentration. 19F-Nano were labeled with fluorescent dyes to easily observe cellular uptake. Using fluorescence microscopy and fluorescence-activated cell sorting analysis (FACS), we verified effective binding between the generated chemical receptors on hMSCs and binding groups on 19F-Nano. Most importantly, we confirmed efficient and fast hMSC-labeling with 19F-Nano using fluorescence and 19F MR imaging. Finally, we explored in vivo tracking of 19F-Nano-labeled hMSCs in mice. We subcutaneously administered 19F (via 19F-Nano)- and dye-labeled cells at a range of concentrations and monitored their fluorescence signals for up to one week after transplantation. We established that about 1,000 hMSCs could be detected in vivo for up to a week. Although we did not monitor ca rdiac remodeling using this approach, we demonstrate that our approach can achieve an effective in vivo detection limit for transplanted 19F-Nano-labeled hMSCs. Therefore, the project goal was achieved.

Our method can provide extensive new ideas for future stem cell19F MRI studies. The PI continues to focus on the proposed research, expand research collaborations with established stem ce ll researchers and clinicians, and compete for research grants to expand stem cell research towards novel stem cell-based imaging and therapy.

Ludovic Zimmerlin Ph.D.
Johns Hopkins University
Mentor: Elias Zambidis, Ph.D.
2013 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Sickle Cell Disease

Genetic Correction of Sickle Cell Disease Human iPSC Converted to a Murine ESC-Like State

This TEDCO funded project has led to the development of novel technology to augment the functional capacity of conventional human pluripotent stem cell (hPSC) cultures. The main aim of this MSCRF support has now been completed and a revised manuscript is currently submitted for publication. Follow-up studies have already been initiated and funded via several supports, including MSCRF. The main goal of this study was to develop a novel and safe chemical approach to convert hPSC to a mouse-like ground state of pluripotency that would permit enhanced gene targeting and differentiation to meet the capacities required for clinical applications, such as the treatment of sickle cell anemia. We have previously published efficient non-viral non-integrating methodologies to reprogram in bulk human blood progenitors (BP) into high-quality iPSC (Park et al. 2012) and recently validated their superior hematovascular capacity when compared with established reprogramming techniques (Park et al 2014). This MSCRF fellowship permitted to identify conditions that stably convert conventional hPSC lines to mouse-like cultures by a high throughput chemical screening (over 120 combinations). Reverted lines acquired high clonal proliferation rates, MEK-ERK independence, bFGF signaling unresponsiveness, JAK-STAT3 and BMP4 signaling dependence, high naive-specific gene expressions, dominant distal OCT4 enhancer usage, global DNA CpG hypomethylation, increased ShMC/SMC ratios, X chromosome activation, decreased lineage specific and somatic donor cell memory gene expression, decreased Class I MHC, increased E-Cadherin expression, decreased glycolytic metabolism, and augmented levels of activated beta-catenin. Stable reversion to an authentic mouse ESC-like naive ground state may improve the utility of primed epiblast-like hPSC, but has only been achieved by others using more complex methods that employ transient transgenesis, promote survival or anti-apoptotic activities to sustain viable cultures or incompletely rewired hPSC by necessitating persistence of pro-primed pluripotency factors. Unexpectedly, our studies revealed variable permissivity to our chemical cocktail to attain complete and authentic reversion that correlated with the degree of baseline lineage-primed gene expression and the fidelity of reprogramming to pluripotency. Most of the BP-iPSC lines that were derived using our stromal activated reprogramming system exhibited high-fidelity pluripotency circuits with fewer epigenomic aberrations and were capable of facile reversion using our minimal cocktail. Non-permissive lines included classical skin fibroblast- and diseased iPSC lines, but we identified a transient treatment combination with two additional small molecules that allowed most of these lines to revert to the naïve state with augmented stability. Our group has previously published the derivation and characterization of the erythropoietic progeny of human preimplantation genetic diagnosis (PGD)-derived hESC and hiPSC lines harboring the homozygous sickle cell disease (SCD) hemoglobinopa thy mutation. We successfully reverted all tested diseased SCD and β-thalassemia PSC lines. The second goal of this MSCRF-funded fellowship was to assess transfection efficiencies and optimize homologous recombination (HR) of naive hPSC lines. Our naïve reversion system could augment hPSC transfection efficiencies reaching (up to 30% using over 15kb large constructs, and follow up studies will evaluate the switch of DNA repair machinery, as well as enhanced HR capacities for distinct diseased models, including SCD. The third goal of this project was to determine the differentiation potency of naïve hPSC lines. We have determined that naïve reversion promoted remarkably robust tri-lineage differentiation in teratoma assays and led to a significantly improved multi-lineage in-vitro differentiation capacity across germ layers, including hematovascular progenitor and primitive endodermal populations.Several new projects will build upon these results and assay the functionality and engraftment capacity of these differentiated populations. In summary, we have introduced a novel strategy that can promote efficient derivation and gene targeting of stable, authentic naive hPSC with significantly improved differentiation potencies and minimal retention of epigenetic donor memory. This technology will have high impact for regenerative medicine, human development biology and the generation of humanized gene targeted animal models of disease. It will offer new opportunities for treating genetic disorders such as sickle cell anemia.

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

Identifying Targets of LRRK2 Translational Regulation in Parkinson's Disease Patient Human Dopamine

LRRK2 kinase activity was linked to protein synthesis through the observation that Parkinson's disease-associated mutations in LRRK2 (e.g. G2019S) that increase it's kinase activity result in elevated fluorescent reporter translation in human ES cell-derived cortical neurons with a concomitant increase in phosphorylation of the LRRK2 kinase substrate Ribosomal protein s15. We found that mutant LRRK2 causes an overall increase in bulk protein synthesis in mutant LRRK2 transgenic Drosophila. To determine whether the converse is true and an inhibition of LRRK2 kinase activity causes a decrease in overall protein synthesis as hypothesized, SH-SY5Y cells were treated with a number of potent and specific LRRK2 kinase inhibitors, including LRRK2-IN-1, CZC-25146, HG-10-102-01 at various doses for 1h along with 35S-methionine to measure global protein synthesis rates. While these compounds effectively inhibit the activity of endogenous wild type and mutant LRRK2, they do not result in a decrease of overall protein synthesis rates. Hence, the original goal of acute LRRK2 kinase inhibition followed by translational profiling to separate out direct translational targets of LRRK2 vs. steady-state alterations following manipulations of LRRK2 expression is not obtainable through the use of LRRK2 kinase inhibitors as originally proposed. Instead, we have focused on characterizing potential LRRK2 translational targets observed to be upregulated in G2019S LRRK2 iPS-derived dopamine neurons relative to control neurons. Our translational profiling indicates that mutant LRRK2 leads to an upregulation in the translation of certain calcium channels, which we have independently confirmed as exhibiting higher expression in post-mortem striatum derived from PD patients carrying the G2019S LRRK2 mutation.

We have begun to grow human iPS-derived dopamine neurons for electrophysiological profiling for basal activity and pace making activity based on the hypothesis that altered channel expression in G2019S LRRK2 carrying neurons may affect these neuron properties. We will also use well characterized calcium channel blockers to observe whether these can prevent hypothesized changes in electrophysiological properties of neurons as well as identified pathological phenotypes of LRRK2 toxicity, such as mitochondrial deficits. If calcium channels can successfully inhibit LRRK2 toxicity in human dopamine neurons carrying the G2019S LRRK2 mutation, this could have significant translational potential as therapy for Parkinson's disease.

Jun Wang, Ph.D.
Phycin, LLC
2014 Exploratory Award Budget: $100,000
Disease Target: Multiple

Recombinant Growth Factors from Algae & their Application in Human Pluripotent Stem Cell Research

The funding period was for one year starting June 30, 2014, and extended to September 30, 2015. Phycin has achieved the research targets that led the project to a significant success. We were the first to express recombinant human growth factors (acidic fibroblast growth factor and basic fibroblast growth factor) from both chloroplast and nuclear genomes of algae, and the expression levels enable economical production of these important recombinant proteins. We further developed proprietary technology to purify the algae derived growth factors to high purity. The purification technology is robust and economical, which is important for the commercial success. Preliminary mitogenic assay of purified bFGF has shown superior activity of the algae derived growth factor over the current market products from other production platform.

Our collaborator at Johns Hopkins University has indicated that a FGF produced by algae system is capable of promoting the growth of human pluripotent stem cells (hiPSC), and is capable of helping hiPSCs remain pluripotency, as evidenced by real-time PCR quantification of the expression of pluripotent genes.The data we have collected so far has clearly demonstrated our proprietary algae system as a viable platform for production of recombinant growth factors that are bacterial endotoxin free, human and animal pathogenic viruses free. The algae derived growth factors are potent in stimulating stem cell proliferation. When scaled up, the cost for the growth factor production can be driven down substantially, comparing to any other current systems. As the commercialization progresses, the stem cell research and application communities will soon benefit from the algae derived growth factors with many sought after advantages.

Wenxia Song Ph.D.
University of Maryland College Park
2012 Exploratory Award Budget: $230,000
Disease Target: Multiple

In Vitro Differentiation of Human Induced Pluripotent Stem Cells Into B-Cells For Modeling Human Diseases

The goal of this project is to explore a new effective method to differentiate human induced pluripotent stem cells (hiPSCs) into the B-lymphocyte lineage in vitro and to apply this in vitro B-cell differentiation process to model B-cell related human diseases.

To pursue these goals, we proposed the following two aims:

Aim 1. To develop a new in vitro culture method for effective differentiation of human iPSCs into the B-celllineage.

Aim 2. To model the X-linked agammaglobulinemia disease by manipulating Btk activity and expression in human iPSCs and derived B-cells.

We have completed the proposed experiments and produced two publications. Another manuscript is in preparation.

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- Seeley-Fa llen, M. K., L. J. Liu, M. R. Shapiro, 0 . 0. Onabajo, T-H Tan, A. Upadhyaya, and W. Song #. 2014. Actin binding protein-1 1inks B-cell receptor to negative signaling pathways. Proc. Natl. Acad. Sci. USA. 111:9881-9886.

During the non-cost extension period, we have generated a better viral vector to introduce shRNA and eDNA of Btk into iPSCs and cells differentiated from iPSCs. We have also established the technique to transfer cells differentiated from iPSCs to humanized mice.

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