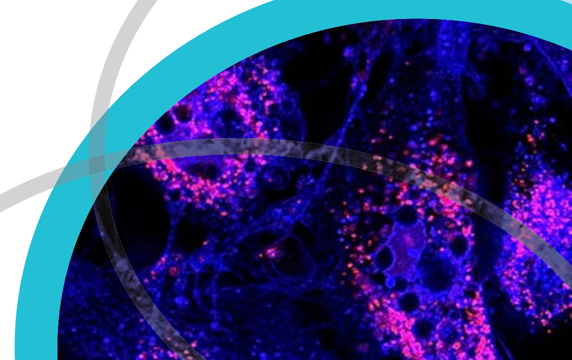
MSCRF
Maryland Stem Cell Research Fund

Maryland
STEM CELL RESEARCH RESEARCH FUND
Annual Report

Promoting State-Funded Stem Cell Research & Cures

Calendar Year
2014



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Maryland Stem Cell Research Commission

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

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

Rachel Brewster, Associate Professor
(Appointed by the University System of Maryland)
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.

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

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

Noel Rose, M.D, Ph.D.
(Appointed by Johns Hopkins University)
Director of the Johns Hopkins Autoimmune Disease
Research Center, MMI & Pathology, & Director of the
Pathobiology Training Program in the School of Medicine.

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

Ira Schwartz, Esq.
Senior Assistant Attorney General & Counsel to the
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 2014 MSCRF Grant Award Recipients

Pre-Clinical Grant Award:

Stephen Wolpe
Orgenesis, Inc.
Autologous Insulin Producing (AIP) Cells for Diabetes

Investigator Initiated Grant Awards:

Curt Civin
University of Maryland, Baltimore (UMB)
Genetic Modification of Sickle Cell Disease in Hematopoietic Stem Cells

Warren Grayson
Johns Hopkins University- School of Medicine (JHU)
PDGF-Modified Fibrin Hydrogels for ASC-Mediated Bone Repair

Ahmet Hoke
Johns Hopkins University- School of Medicine (JHU)
Genetic and Functional Comparison of ESC & iPSC Derived Neural Crest Stem Cell Lineages

Hai-Quan Mao
Johns Hopkins University- School of Medicine (JHU)
Nanofiber Matrix to Enhance Human Retinal Ganglion Cell Generation

Ke Ren
University of Maryland, Baltimore (UMB)
Mesenchymal Stem Cells for Chronic Pain Therapy

Hongjun Song
Johns Hopkins University- School of Medicine (JHU)
Synaptic Mechanisms Underlying Major Psychiatric Disorders

Elias Zambidis
Johns Hopkins University- School of Medicine (JHU)
Ground State Naive Human iPSC Banks for Vascular Regenerative Medicine

Exploratory Grant Awards:

Jonathan Dinman
University of Maryland, College Park (UMCP)
Directed delivery of Therapeutic RNAs into Hematopoietic Stem-Progenitor Cells

Stephen Eacker
Johns Hopkins University- School of Medicine (JHU)
Translational Targets of mTOR in hNPC Development

Sonia Franco
Johns Hopkins University- School of Medicine (JHU)
Induced Pluripotent Stem (iPS) Cell-Based Approaches for Modeling and Treating Ataxia-Telangiectasia

Marta Lipinski
University of Maryland, Baltimore (UMB)
Modeling Parkinson‘s Disease Function of the PARK10 Gene USP24 in Human iPS Cells

A-Lien Lu-Chang
University of Maryland, Baltimore (UMB)
The Effects of Histone Deacetylation & DNA Demethylation on Somatic Cell Reprogramming

Vasilki Machairaki
Johns Hopkins University- School of Medicine (JHU)
Induced Pluripotent Stem Cell Strategies to Model Alzheimer‘s Disease

Nicholas Maragakis
Johns Hopkins University- School of Medicine (JHU)
IPSC-Derived Neurons from Amyotrophic Lateral Sclerosis Patients To Study Disease Progression

David Nauen
Johns Hopkins University- School of Medicine (JHU)
Investigating Mechanisms of Epileptogenesis Using Human Induced Pluripotent Stem Cells

Tea Soon Park
Johns Hopkins University- School of Medicine (JHU)
Treatment of Diabetic Retinopathy with Human iPSC-Derived Vascular Progenitors

Venu Raman
Johns Hopkins University- School of Medicine (JHU)
Manipulating Intestinal Stem Cells To Mitigate The Effects Of Inflammatory Bowel Diseases

Feyruz Rassool
University of Maryland, Baltimore (UMB)
Efficiently Reprogramed Cells with a MYC Signature Display High Fidelity Repair of DNA Damage

Joseph Stains
University of Maryland, Baltimore (UMB)
The Role of the beta-catenin Signaling Cascade in the Skeletal Phenotype of Hutchinson-Gilford Progeria Syndrome

Jun Wang
Phycin, LLC
Recombinant Growth Factors from Algae & Their Application in Human Pluripotent Stem Cell Research

Jiangyang Zhang
Johns Hopkins University- School of Medicine (JHU)
Magnetic Resonance Imaging of Myelination by Transplanted Glial Restricted Precursor Cells

Jiangyang Zhang
MedStar Health Research Institute
Enhancing the Incorporation of Bone Allograft with Circulating Mesenchymal Stem Cells

Post-Doctoral Fellowship Grant Awards:

Tong Ma
Johns Hopkins University- School of Medicine (JHU)
Investigating the Role of a Mental Disorder Risk Gene in the Development of Human GABAergic Neurons

Manoj Kumar
Johns Hopkins University- School of Medicine (JHU)
Human Dopaminergic Neuronal Loss Due To Parkin Insufficiency: Relevance to Parkinson’s Disease

Sang Hoon Kim
Johns Hopkins University- School of Medicine (JHU)
Modeling and Characterization of Double Cortex Syndrome Using iPSCs and Cerebral Organoids

Hui Lin
Johns Hopkins University- School of Medicine (JHU)
Bioengineering A Lacrimal Gland Using Human Lacrimal Stem Cells

Lipeng Tian
Johns Hopkins University- School of Medicine (JHU)
Human Stem Cell based Model of Alcoholic Liver Disease for Regenerative Therapy

Anna Jablonska
Johns Hopkins University- School of Medicine (JHU)
Genetic Engineering of Glial Progenitor Cells for Improved Intra-Arterial Targeting In Stroke

Ian Martin
Johns Hopkins University- School of Medicine (JHU)
Identifying Targets of LRRK2 Translational Regulation in Parkinson’s Disease Patient Human Dopamine

Raju Khatri
University of Maryland, Baltimore (UMB)
Increasing the Replicative Lifespan and Quality of Adult Mesenchymal Stem Cells

Closed: 2011 Investigator Initiated Award:

John Laterra
Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
Regulation of Neural and Neoplastic Stem Cells by Kruppel-like Transcription Factors

Closed: 2012 Exploratory Awards:

Tami Kingsbury
University of Maryland, Baltimore (UMB)
MicroRNAs and Control of Quiescence and Pluripotency

Chulan Kwon
Johns Hopkins University- School of Medicine (JHU)
Membrane Notch Control of Human Cardiovascular Progenitors

Sivaprakash Ramalingam
Johns Hopkins School of Public Health (JHSPH)
Functional Correction of hiPSCs with Homozygous Sickle Cell Disease Mutation Using Engineered ZFNs/TALENs

Antony Rosen
Johns Hopkins University- School of Medicine (JHU)
Using hESCs to Define Novel Scleroderma Autoantigens in Stem Cells and Vascular Progenitors

Mingyao Ying
Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
Highly Efficient Conversion of Human Stem Cells to Dopaminergic Neurons by Proneural Transcription Factor Atoh1

Closed: 2012 Post-Doctoral Fellowship Awards:

Ola Awad
University of Maryland, Baltimore (UMB)
Role of Autophagy Dysregulation in the Development of Neurodegeneration Using iPSC Model of Gaucher's Disease

Su Mi Choi
Johns Hopkins University- School of Medicine (JHU)
In collaboration with Cellomics Technology, LLC
Patient Specific Stem Cell based In Vitro Model of Liver Cirrhosis

Eunchai Kang
Johns Hopkins University- School of Medicine (JHU)
Modeling of Major Mental Disorders Using Human Induced Pluripotent Cells Derived from Patients With a Defined Disc1 Mutation

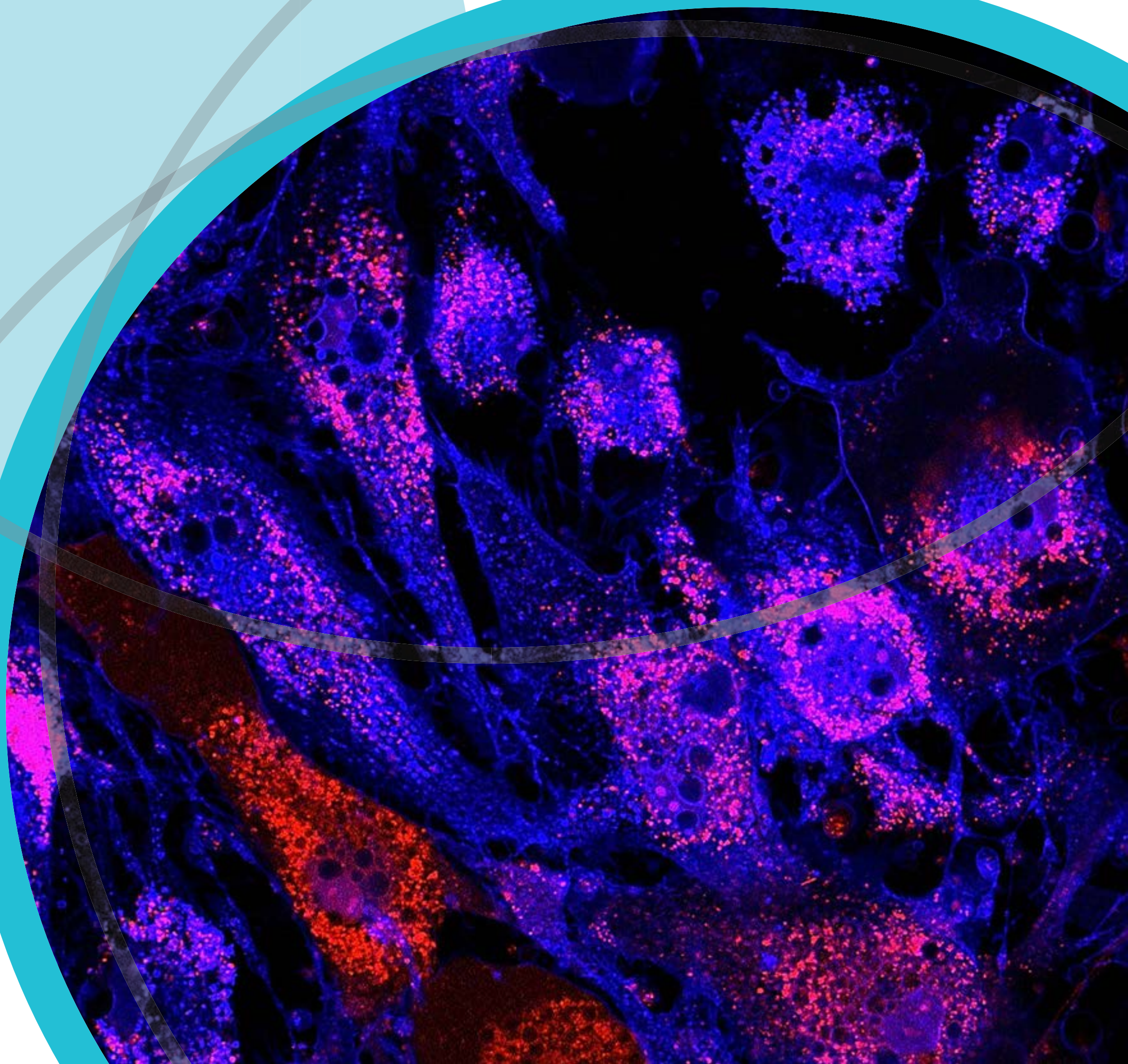
Changmei Liu
Johns Hopkins University- School of Medicine (JHU)
Small RNA Regulation Of GSK3 Expression Modulates Human Neural Stem Cells Proliferation and Differentiation

Georgia Makri
Johns Hopkins University- School of Medicine (JHU)
Patient-Specific iPSCs for Modeling and Treatment of Rett Syndrome

Yi-Lan Weng
Johns Hopkins University- School of Medicine (JHU)
Effects of DNA Methylation Reprogramming in Axonal Plasticity and Regeneration

Yaxue Zeng
Johns Hopkins University- School of Medicine (JHU)
Characterizing the Role of Active DNA Demethylation in Reprogramming of Human Somatic Cell into Stem Cells

2014 Pre-Clinical Grant Award:



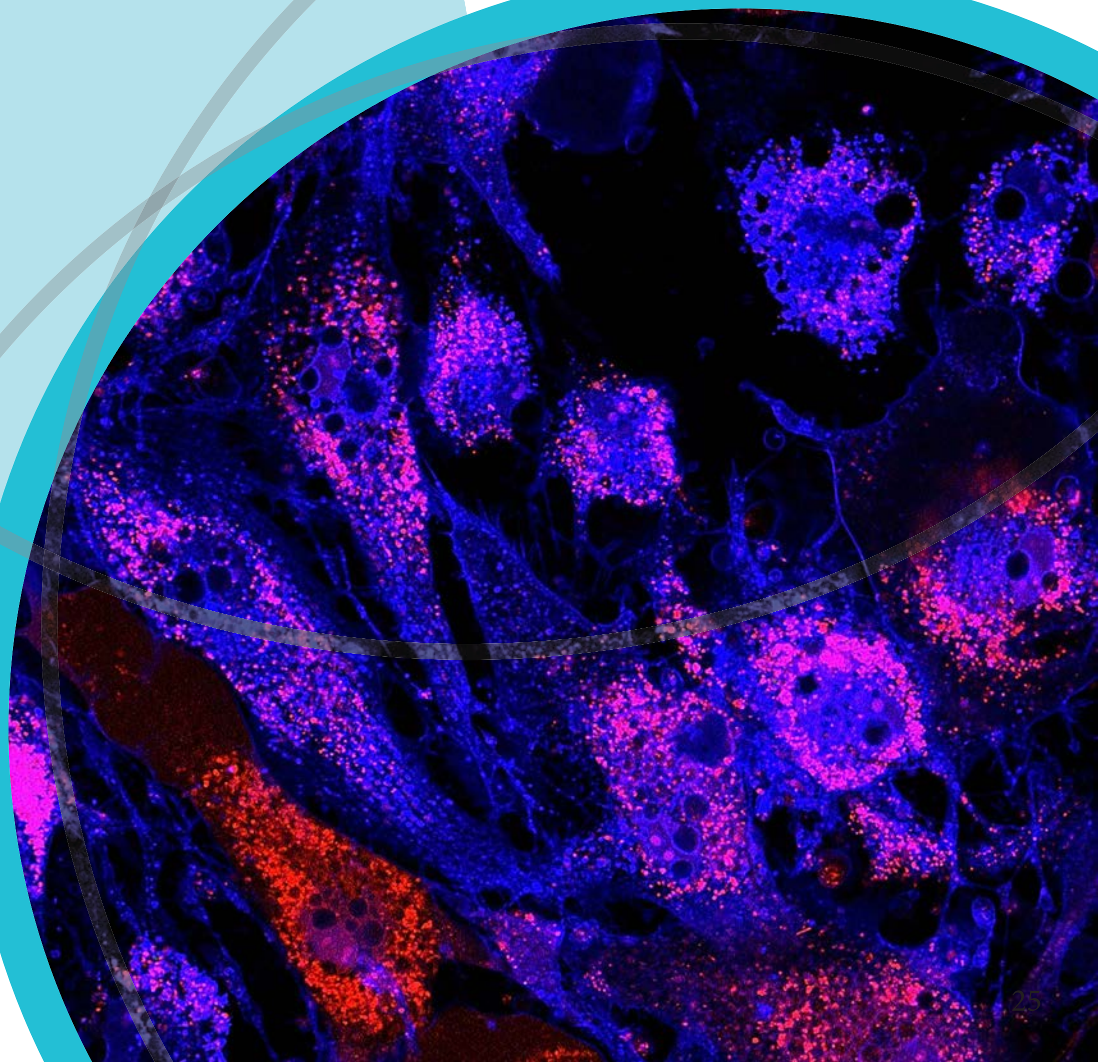
Stephen Wolpe, Ph.D.
Orgenesis, Inc.
Award Amount: $406,431
Disease Target: Diabetes

Autologous Insulin Producing (AIP) Cells for Diabetes

Orgenesis, Inc. (OTCBB:ORGS) is a publically traded, development stage biopharmaceutical company based in the Innovation Center in Germantown, Maryland, that is developing a cell and gene therapy for diabetes. Diabetes is a disease caused by the inability to properly regulate blood sugar (glucose) levels. Insulin is the major hormone that regulates glucose levels and diabetes involves inadequate responses to and/or production of insulin. There are two major types of diabetes: Type 1 diabetes (T1D), an autoimmune disease where the patient's own immune cells kill the insulin producing islets in the pancreas, and Type 2 diabetes (T2D), where the patient's islets fail to produce sufficient amounts of insulin. Orgenesis' lead product targets insulin dependent diabetes mellitus (IDDM) i.e., those diabetics (T1D or T2D) who are insulin dependent. This is a large population of over 25 million people in the US, or about 8% of the US population: Diabetes Type I (100% are insulin dependent) • Diabetes Type II patients who use insulin (about 50% of the diabetic population) Orgenesis has developed a technology that uses a population of mesenchymal stem cells (MSC) derived from the patient's own liver to replace the insulinproducing cells that are missing in IDDM patients. Because they are adult cells derived from the patient's own liver these cells are not considered ethically questionable. The process involves taking a standard liver biopsy from the patient, growing the cells in tissue culture, adding genes to the cells that induce differentiation into insulin producing cells, and then implanting them back into the patient's liver. The process is similar to an existing clinical protocol, called the Edmonton protocol, that implants islets into the livers of patients with Type 1 diabetes.

This procedure restores the ability of the patient to control glucose but requires the persistent use of powerful drugs to suppress the immune system to keep it from rejecting the islets (which are foreign to the patient since they come from an unrelated donor). In contrast, since the Orgenesis protocol uses the patient's own cells, these drugs are not needed and the patient is spared their toxic side effects. Global organizations such as the World Health Organizations and the International Diabetes Federation estimate that the global burden as health care expenditure to treat and prevent diabetes and its complications exceeded $400 billion in 2012 (approximately 10% of total global expenditure for health). The disease is of major interest to the State of Maryland, where the prevalence of diabetic adults has grown from 6.8% in 1999 to 8.7% in 2008, which continues to be above the national level and where the development of a new pharmaceutical company will lead to increased jobs and tax revenues. From an investment point of view, the Orgenesis process is particularly attractive to the State because it requires the development of specialized manufacturing techniques and personnel that are not easily transferred to another location after commercial launch. The Orgenesis process is a novel regenerative medicine approach to treating diabetes. Traditional diabetes drugs either sensitize the body to insulin action or supply insulin itself. As a result, these treatments do not address the core defect in diabetes, which is impaired activity or destruction of the pancreatic islet cells that produce insulin in the body. As pancreatic islets do not divide, stem cell replacement therapies approaches must be used to generate sufficient numbers of islets to cure a diabetic patient. Orgenesis is currently developing a clinical grade procedure and is planning to conduct its first trials in Europe, to be quickly followed by trials in US. It established the company in Maryland and had preliminary discussions with FDA. The current application is aimed to establish the Orgenesis process in Maryland and to conduct the experiments FDA suggested will be needed before running US clinical trials.

2014 Investigator Initiated Grant Awards:



Curt Civin, M.D.
University of Maryland, Baltimore
Award Amount: $690,000
Disease Target: Sickle Cell Anemia & Other

Genetic Modification of Sickle Cell Disease in Hematopoietic Stem Cells

In concept, the medical problems of Sickle Cell Disease would be solved permanently by a selftransplant of the patient's own blood-forming stem cells which had been "gene-edited" to prevent them from sickling by recently developed recombinant DNA techniques that would either: Correct the classic Sickle Cell Disease mutation in the patient's blood-forming stem cells—Dr. Donald Kohn's California Institute of Regenerative Medicine (CIRM) project (Beta-Globin Gene Correction of Sickle Cell Disease in Hematopoietic Stem Cells) investigates using a "Zinc Finger Nuclease" technique to actually correct the mutation in the Sickle Cell Disease patient's DNA that produces mutant sickle hemoglobin—or B. Disrupt a regulatory region of DNA (called "the BCL11A erythroid enhancer"—
Patients with the Hereditary Persistence of Fetal Hemoglobin (HPFH) syndrome have reduced severity of Sickle Cell Disease (and â-thalassemias) because sickling of their mutant hemoglobin is prevented by their high red blood cell levels of fetal hemoglobin that persist lifelong in people with this syndrome. It has been shown recently that HPHF is due to a harmless variation in their DNA that disrupts (i.e. effectively deletes) a DNA control element called the BCL11A erythroid enhancer. In most humans, high levels of BCL11A keep fetal hemoglobin levels low in adulthood. Disrupting the BCL11A erythroid enhancer by gene-editing of blood-forming stem cells should "copy" the key features of HPHF, by lowering BCL11A levels and, in turn, fetal hemoglobin in red blood cells. Self-transplant of such gene-edited blood-forming stem cells to Sickle Cell Disease patients should generate red blood cells that cannot sickle. To complement and enhance Dr. Kohn's CIRM project investigating option A, we propose to collaboratively investigate option B, disrupting the BCL11A erythroid enhancer in human bloodforming stem cells by a new technique called the Cas9 system. Specific Aim 1 will optimize the Cas9 strategy to efficiently disrupt the BCL11A erythroid enhancer element sufficiently to lower BCL11A and raise fetal hemoglobin in human red blood cells. Specific Aim 2 will quantitate the toxicity and leukemogenicity of Cas9-disruption of the BCL11A erythroid enhancer in blood-forming stem cells. We predict that our collaborative MSCRF-CIRM studies will provide a foundation for clinical self-transplant of gene-edited blood-forming stem cells for Sickle Cell Disease.

Warren Grayson, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $689,743
Disease Target: Critical-Sized Bone Defects

PDGF-Modified Fibrin Hydrogels for ASC-Mediated Bone Repair

The treatment of large craniomaxillofacial and orthopaedic bone defects due to congenital defects, trauma or cancer resection remains a huge clinical challenge. There are approximately one million fractures requiring bone transplantation annually in the US. This accounts for 20% of the global use of bone grafts and incurs an annual economic burden of $3 billion. Yet, there remains no satisfactory solution for critical-sized, geometrically complex bone defects, which result from trauma, tumor resections, and congenital malformations. In order to stabilize patients with these deformities, surgeons are forced to rely on expensive stopgap technologies that do not recoup quality of life, do not suitably resolve the defect, and that are often burdened with multiple, long term complications imposing further economic burden due to additional procedures, longer/repeated inpatient stays, and medication. The repair of these bone defects significantly impacts the U.S. health care system and there is a clear clinical need for the development of a method to regenerate vascularized bone to provide the restitution of original 3D configuration and restore patients with craniofacial and orthopedic bone defects back to their normal function and appearance. The current gold standard for repair, autologous bone grafts, results in significant donor-site morbidity and is limited by the amount of useful bone that can be harvested. Stem cell-based, tissue engineering (TE) approaches provide a novel alternative for the treatment of bone defects. Yet TE techniques have so far failed to deliver therapeutically since they are technically challenging (rendering them unsuitable for clinical environments) and they lack commercially viability. We propose to develop a novel cell/biomaterialbased approach that is scientifically sound, capable of overcoming practical and regulatory obstacles, and has the potential to be commercially viable. Our previous in vitro studies have demonstrated that in the presence of physiological concentrations of platelet-derived growth factor (PDGF), human adipose-derived stem/stromal cells, the major cellular component of the stromal vascular fraction (SVF) of cells harvested from lipoaspirate, can form interconnected vascular networks surrounded by dense bone nodules. We will harness these SVF-PDGF interactions to engineer vascularized bone grafts that are suitable for treating large, nonhealing bone defects. In this study, we propose to modify fibrin hydrogels to enhance the cell and growth factor binding capabilities. We will encapsulate SVF and PDGF in the hydrogels and inject into 3D-printed, porous scaffolds and immediately implant into non-healing bone defects in immunocompromised rats. In this study, we will rigorously characterize the potential of cells to form vascularized bone in the presence of soluble growth factor (Aim 1), assess the synergistic effects of enhanced cell- and growth factor-binding to fibrin on tissue development (Aim 2), and evaluate the influence of these factors to mediate robust vascularized bone regeneration (Aim 3). We will assess functional and histological outcomes at 12 weeks following surgeries. This proposed approach offers key scientific and practical advantages over current methods: (1) SVF can be harvested and used intra-operatively. By generating bone grafts with intrinsic vascular networks, we will overcome a critical barrier to successful integrations of functional bone grafts. (2) Functionalized hydrogels and 3D-printed scaffolds can be commercialized and made available offthe- shel(3) Porous scaffolds can be made into anatomical shapes based on CT image analysis to make customized grafts for clinical applications. The combination of SVF, PDGF, fibrin and 3Dprinted scaffolds has been selected for the scientific and clinical relevance of each of these components, their ability to work synergistically to facilitate the development of a complex tissue graft, direct and significant clinical impact.

Ahmet Hoke, M.D., Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Peripheral Nerve

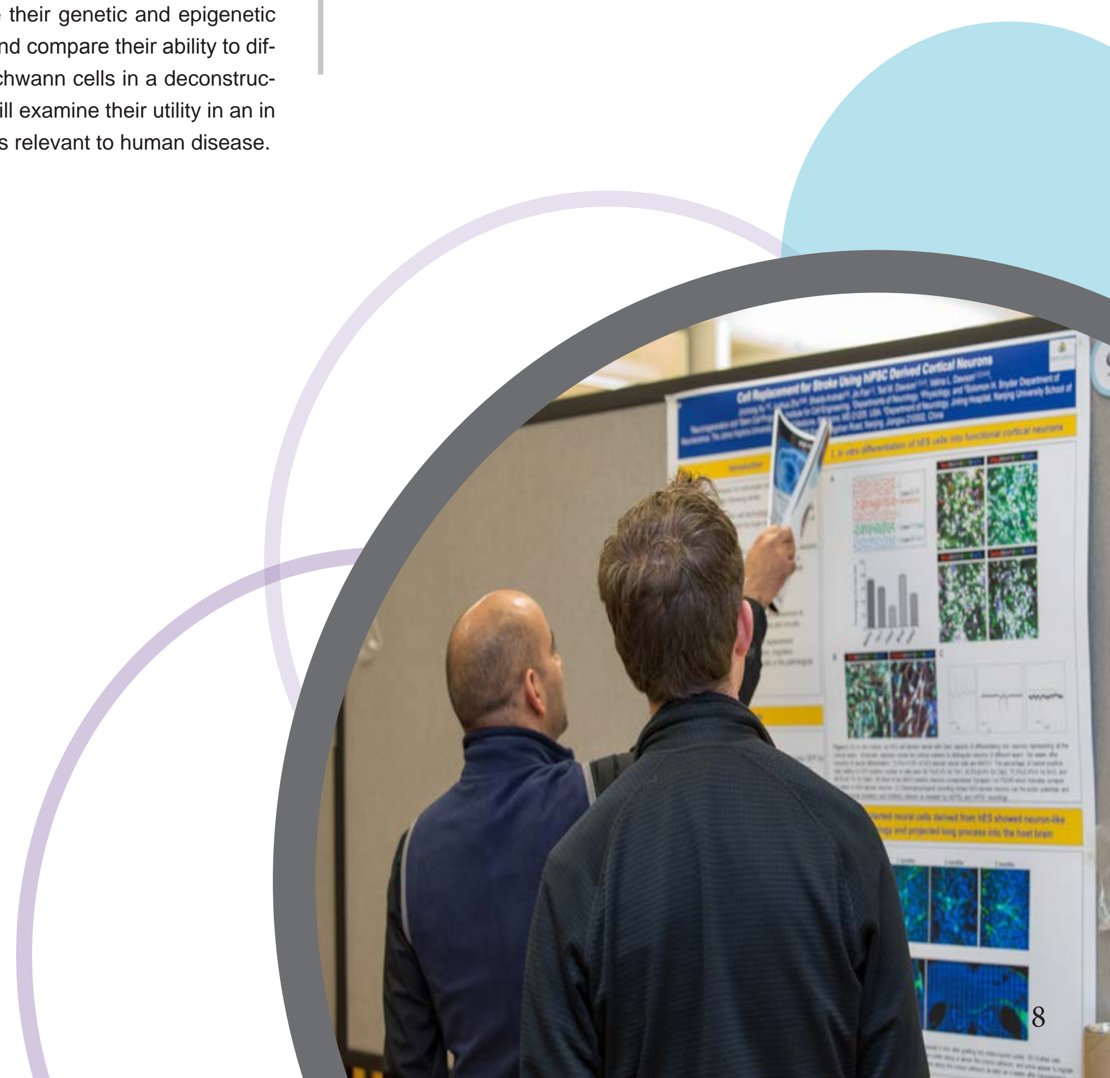
Genetic and Functional Comparison of ESC- and iPSC-Derived Neural Crest Stem Cell Lineages

Injury to the peripheral nervous system (PNS) results in significant morbidity despite the inherent ability of peripheral nerves to regenerate. This regenerative failure has multiple causes but one of the key factors is atrophy and loss of the supporting cells in the peripheral nerves called, Schwann cells. Peripheral nerve trauma offers an ideal clinical target to develop cell based therapies because, robust outcome measures are available in multiple animal models that mimic human disease and accessibility of the target tissues for cellular transplantation during surgical repairs of injured nerves. The proposed project takes advantage of this model system to test the hypothesis that human induced pluripotent stem cells (hiPSC) are comparable to human embryonic stem cells (hESC) in terms of their potential to differentiate into functional Schwann cells and help repair chronic peripheral nerve injuries. Although, comparisons between hiPSCs and hESCs have been done in other disease models, none have been carried out to the same rigor proposed in this application with regards to comprehensiveness of the methods used to generate their progenies, outcome measures used and the disease model tested. We will accomplish this out by generating NCSCs and Schwann cells from both sources under the same laboratory conditions and explore their genetic and epigenetic profiles in aim 1. In aim 2, we will examine and compare their ability to differentiate into fully functional myelinating Schwann cells in a deconstructed in vitro assay system and in aim 3, we will examine their utility in an in vivo chronic nerve regeneration model that is relevant to human disease.

Hai-Quan Mao, Ph.D.
Johns Hopkins University,School of Medicine
Award Amount: $600,000
Disease Target: Traumatic Optic Nerve

Nanofiber Matrix to Enhance Human Retinal Ganglion Cell Generation

Retinal ganglion cells play a pivotal role in the transmitting visual information from the retina to the brain through their axon bundle in the optic nerve. Damage to the optic nerve results in permanent vision loss. Transplantation of retinal ganglion cells has been proposed as a potential cell replacement therapy for optic nerve regeneration. However, there is no available method for generating functional retinal ganglion cells in sufficient quantity and quality. We propose to develop a nanomaterial-based platform that can allow efficient generation of human retinal ganglion cells from pluripotent stem cells and test the function of these cells. This study will provide a robust cell source for generating optic nerve-like tissue structure and enable studies of cell-based therapies to replace damaged retinal ganglion cells and their axons in the optic nerve, and contribute to the ultimate goal of reestablishing functional vision in patients made blind by RGC death due to injury and disease.



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

Mesenchymal Stem Cells for Chronic Pain Therapy

Chronic pain affects millions of people and is a major health problem. It costs the US over $500- $650 billion each year in health care and lost productivity. It is also recognized in the State of Maryland that chronic intractable pain is a debilitating condition and may often be inadequately treated. The current treatment for chronic pain conditions such as neuropathic and musculoskeletal pain is unsatisfactory and there is an urgent need for searching and developing alternative and effective pain therapy. Multi potent stromal cells, or mesenchymal stem cells, can be derived from a variety of sources such as bone marrow, adipose tissues, the dental pulp, the umbilical cord and muscle. They are relatively easy to isolate and expand in culture, possess immune regulatory properties, and secrete trophic mediators that may favor tissue repair and regeneration. In recent years, mesenchymal stem cell medicine has progressed beyond pursuing structural cell repair and replacement, in recognition of sophisticated interactions between the implanted cells and the host and other potential therapeutic benefits. mesenchymal stem cell have shown encouraging results in treating neurological disorders in clinical studies. Interestingly, mesenchymal stem cells appear to have potential to treat chronic pain conditions. We have initiated studies on the effect of bone marrow derived mesenchymal stem cells on pain. In rat models of persistent pain induced by nerve or tissue injury, long lasting pain hypersensitivity developed, resembling some features of clinical chronic pain conditions. Infusion of rat bone marrow derived mesenchymal stem cells produced long term attenuation of behavioral pain and the effect involved activation of opioids in the body. The cellular mechanisms of the effect of bone marrowderived mesenchymal stem cells on pain are still elusive. Studies suggest that the infused bone marrow derived mesenchymal stem cells produce their therapeutic effects through secretion of chemical mediators that interact with the body's immune system. We hypothesize that bone marrow derived mesenchymal stem cells produce the pain relieving effect through immune interactions and subsequent activation of endogenous pain modulation. We propose a series of experiments to test this hypothesis in preclinical persistent pain models by a combination of biomedical approaches. We will first characterize the effects of human bone marrow derived mesenchymal stem cells. We will then test the hypothesis that up regulation of opioid receptors and pain reduction by bone marrow derived mesenchymal stem cells is mediated by immune mediators. Our results will provide the cellular mechanisms of bone marrow derived mesenchymal stem cells in pain relief and prompt translating this approach into clinical settings. The findings will contribute to the advancement of stem cell medicine and formulation of novel stem cell based pain management.

Hongjun Song, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Schizophrenia & Depression

Synaptic Mechanisms Underlying Major Psychiatric Disorders

Patients with psychiatric illnesses, such as schizophrenia, bipolar disorders and major depression, suffer from social and emotional dysfunction and cognitive deficits. Cumulative evidence supports a model that dysregulated neurodevelopment with altered structural and functional connectivity contributes to the development of neuropsychiatric disorders. One major hypothesis suggests that schizophrenia is a disease of synapses. In the context of a neurodevelopmental model, it is proposed that impaired mechanics of synaptic transmission in specific neural circuits during childhood and adolescence ultimately results in altered synapse formation or pruning, or both, which manifest in the clinical onset of the disease. This attractive hypothesis has received support from analyses of postmortem patient brain samples. However, direct evidence is missing and the underlying mechanism is not well understood. While a number of risk genes have been identified from human linkage and genetic association studies, how disruption of any of these genes affects synapse function that can lead to pathogenesis of mental illnesses is a major unanswered challenge. Current available treatments for psychiatric illnesses mostly target symptoms, not the causative pathophysiology. DISC1 (Disrupted-in-schizophrenia 1) is one of the best-supported genetic loci associated with increased risk for major mental illness, identified in multiple studies. In one American Pedigree (Pedigree H), many members carry a four-nucleotide deletion in the DISC1 gene, the majority of whom have been diagnosed with schizophrenia, schizoaffective disorders and major depression. Our laboratory has been working to understand the role of DISC1 in regulating neuronal development using rodent models for the past decade. During the past four years, we have generated and characterized multiple induced pluripotent stem cell (iPSC) lines from Pedigree H and we have generated different types of isogenic lines via genetic editing. Building upon substantial preliminary results from analyses of these iPSC lines, the current project will take advantage of human stem cell technology using human neurons derived from patient iPSCs and isogenic lines to test the following hypothesis: Gain-of-function mutation of risk genes for mental disorders can affect synaptic function via transcriptional dysregulation in human neurons. We will also perform a novel pathway-centric high-throughput screen to identify hits that may ameliorate defects in human neurons. In addition, we will develop a novel in utero transplantation model for in vivo analysis. Our study may lead to a better understanding of the role and mechanism of major risk genes for mental disorders in human neuronal development and shed light onto the etiology and pathogenesis of schizophrenia and related major mental illnesses. Importantly, our study may lead to the development of novel therapeutic treatments for psychiatric disorders based on rational design and hypothesis-driven investigation of relevant cellular pathophysiology.

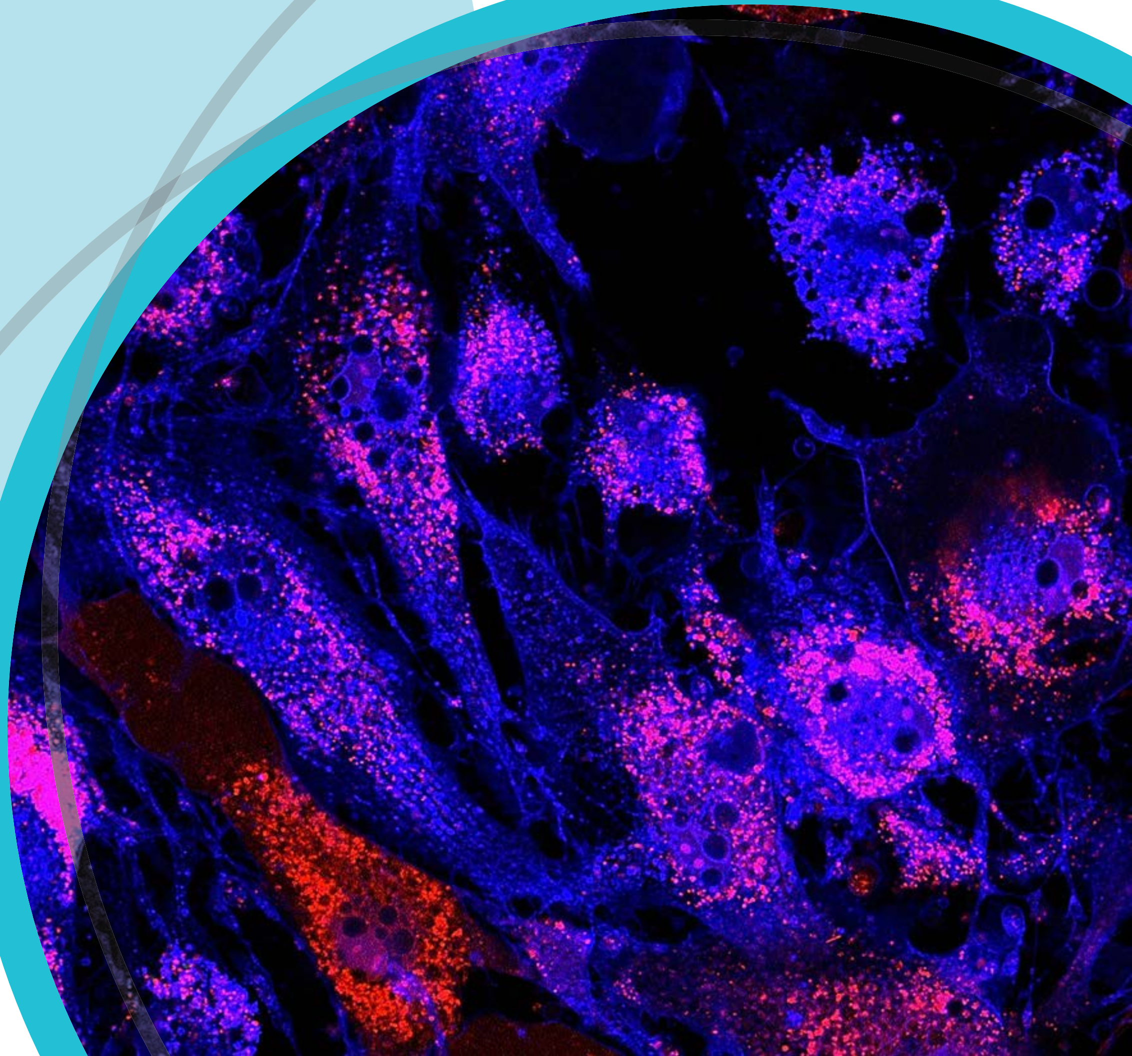
Elias Zambidis, M.D., Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Vascular Disease

Ground State Naive Human iPSC Banks for Vascular Regenerative Medicine

Stem cell treatments for ischemic disorders such as myocardial ischemia, cerebrovascular stroke, sickle cell disease, and diabetic retinopathy ultimately require not only the regeneration of damaged myocardium, brain, hematopoietic, and retinal tissues, but also the reconstruction of the defective vascular niche that instigated the initial disease to begin with. The human vasculature normally arises from highly prolific embryonic angioblasts or vascular progenitors (VP) that differentiate into vascular endothelial cells and pericytes during early development. Such prolific embryolike VP are rare or nonexistent in the adult. Furthermore, although circulating adult endothelial progenitor cells (EPC) have been proposed for vascular cellular therapies, such EPC are not only limited in multipotency and expansion, but also functionally defective in diseases such as diabetes. If ischemic acellular capillaries could be efficiently repaired with autologous or HLAmatched embryonic vascular/pericytic progenitors, end stage vascular diseases such as diabetes and cardiac ischemia could be reversed. One solution would be to differentiate human induced pluripotent stem cells (hiPSC) into VP that possess prolific embryo-like endothelial mesenchymal pericytic potential for regenerating diseased tissues. However, this goal is currently limited by the poor efficiency and variability of directed vascular differentiation from hiPSC. It will be necessary to first generate high quality clinical grade hiPSC via safer non integrating, nonviral derivation methods. In this project, we will accomplish this goal by efficiently reprogramming a patient's own blood cells to a novel high quality state of pluripotency called the "naïve ground state" that resembles mouse embryonic stem cells (ESC). Naïve human iPSC (NhiPSC) possess more versatile abilities than conventional human ESC and hiPSC including more rapid expansion in culture, greater differentiation potencies, and a higher capacity to be genetically manipulated via a method called "gene targeting" (which specifically and safely deletes or adds mutations into the genome of stem cells via a process called homologous recombination). We will generate and differentiate these high quality NhiPSC, and specifically test for their capacity to undergo gene targeting and to produce functional embryonic VP and retinal photoreceptors for future cell therapies of blinding ischemic retinal diseases. Finally, we will address the important issue of the ultimate costeffectiveness of individualized hiPSC therapies by establishing a bank of clinicalgrade, HLAdefined "universal donor" NhiPSC. We propose that complementation of the current paradigm of individualized patientspecific hiPSC approaches with such a universal donor NhiPSC bank can alternatively serve the greater needs of patients that require immediate regeneration of a variety of diseased tissues.

MSCRF
Maryland Stem Cell Research Fund

2014 Exploratory Grant Awards:



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

Directed Delivery of Therapeutic RNAs Into Hematopoietic Stem-Progenitor Cells

Bone marrow or umbilical cord blood transplant (BMT) has radically transformed the practice of medicine, giving hope to patients suffering from a wide range of blood related diseases. These include leukemias and lymphomas, multiple myeloma and other plasma cell disorders, severe aplastic anemia, myelodysplastic syndromes and other hematopoietic disorders including sickle cell disease and thalassemias, inherited immune disorders, and some inherited metabolic disorders. BMT relies on the ability to harvest, expand, and manipulate hematopoietic stemprogenitor cells (HSPCs), a special type of cell that is able to generate for a lifetime all of the different specialized cells found in blood. Unfortunately, it is difficult to obtain sufficient numbers of HSPCs from a single donation of human bone marrow or cord blood. A major challenge facing researchers and clinicians is to devise ways to increase the numbers of HSPCs outside of the human body so as to produce from a single donation the large numbers required for multiple successful BMT therapies. Similar problems pertain to to generating the huge numbers of mature red blood cells (erythrocytes) needed for transfusions anemias. The Civin laboratory has shown that delivery of specific small RNAs into HSPCs can stimulate them to develop generate larger numbers of maturing red blood cells. The problem is that current methods used to deliver these RNAs are inefficient, nonspecific, toxic, labor intensive, and potentially cancer causing. In collaboration with Birich Technologies, a Marylandbased small startup biotechnology company, the Dinman laboratory (University of Maryland College Park) has developed a novel tool that that enables safe, efficient and specific delivery of therapeutic RNA molecules into cells. The technology is generically called ChemoARP. Different types of ChemoARPs can deliver RNAs into different types of cells. In this application, the Dinman laboratory will test the ability of two different ChemoARPS to deliver specific RNA molecules into a different human hematopoietic cell lines. One set of experiments will be performed in collaboration with the Civin laboratory (University of Maryland Baltimore) to develop these ChemoARPs to specifically stimulate amplification of red blood cells for therapeutic applications. In addition, the technology developed herein will be used as a guide to enable similar genetic manipulations of difficulttotransfect cell lines and primary cells. The results of the proposed research will constitute novel technological advances impacting both laboratory and clinical use. These studies will also help to generate tangible products for a Marylandbased biotechnology company, train new stem cell researchers, and create well paying, high technology jobs in the State of Maryland.

Stephen Eacker, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Autism Spectrum Disorders, Schizophrenia

Translational Targets of mTOR in hNPC Development

Abnormal nervous system development underlies numerous neurological and neuropsychiatric disorders. These brain abnormalities can arise from errors in the genetic program of neuronal cells themselves or can be the result of improper receipt of extrinsic developmental signals from other, non-neuronal cells. One potent extrinsic signaling factor is the insulin-like growth factor (IGF), which acts to promote growth and division of neural cells. IGF promotes the activation of a protein called the mechanistic target of rapamycin (mTOR), stimulating the synthesis of new proteins. Little is known about what proteins are synthesized as a result of mTOR activation in developing human nervous system, but defects in mTOR activation are associated with syndromic forms of autism. We have developed a method of generating human neurons from hES and hIPS cells that recapitulates the many of diverse neuronal cell-types found in the human cortex. We have demonstrated that the development of these complex cultures of human neurons requires IGF and mTOR signaling at the neuronal precursor stage. Our goal is to identify the proteins synthesized as a result of activation of the IGF/mTOR signaling pathway in neural precursors and to determine how they promote the development of human cortical neurons. Having achieved the objectives, we will have identified a molecular mechanism for IGF signaling that promotes neuronal development, an essential step for understanding how disruptions in IGF and mTOR signaling can alter the diversity of neuronal cell types that are though to result in neuropsychiatric conditions like autism and schizophrenia.

Sonia Franco, M.D., Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Peripheral Nerve

Induced Pluripotent Stem (iPS) Cell-Based Approaches for Modeling &Treating Ataxia-Telangiectasia

The main goal of this application is to develop novel human stem cell-based approaches for the treatment of the genetic syndrome AtaxiaTelangiectasia (AT). AT is a devastating disease that typically manifests early in childhood with loss of muscle control due to neuronal degeneration, recurrent infections due to alterations in the immune system and high predisposition to cancer. It is already known that AT is caused by mutations in the locus encoding the protein ATM (Ataxia Telangiectasia Mutated) at human chromosome 11q22. Therefore, patients suspected to have this disease are readily diagnosed using genetic testing. Once the diagnosis is made, AT patients are followed in multidisciplinary clinics by a team of neurologists, immunologists, oncologists and other specialists, such as the Johns Hopkins AT Clinic in Maryland. Due to the lack of any treatments, the current standard of care consists of supportive measures. As a result, most AT patients die from infection or cancer in the second or third decade of life. There is therefore a pressing need to develop novel approaches to increase the quality of life and lifespan of children and young adults suffering from AT. We believe that the recent development of technologies to generate induced pluripotent stem cells (iPSCs) from patients' skin or blood cells, coupled with emerging genome engineering tools, provides such an opportunity. Therefore, the overall goal of this application is to start to explore how to best apply the promise of regenerative medicine to AT patients. In Aim 1, we propose to take skin cells from patients and make patienttailored iPSCs ("autologous iPSCs"). This is important because not all AT patients are equal. Depending on the underlying mutation, some AT patients have more severe disease than others, or their disease may be particularly severe in certain organs. This very complex clinical spectrum makes difficult for doctors to individualize therapies and to provide genetic counseling to the affected families. To address this problem, the proposed work will test the hypothesis that the iPSCs generated from patients with different clinical symptoms and severity will recapitulate the original features of the disease, serving as a "surrogates" for patient's cells. For example, iPSCs originated from patients that are very sensitive to radiation will also be very sensitive to radiation, while those originating from patients that are less sensitive will be less so. If we find this to be the case, autologous iPSCs may be generally used in the future to test biomarkers or treatments for AT patients. In addition, experiments in Aim 2 will start to explore the possibility of treating the symptoms of AT by replacing the damaged cells with diseasefree cells.

Currently, a major challenge to the use of regenerative medicine for the treatment of AT is that AT cells reprogram very poorly when compared to normal cells. Moreover, the rare iPSCs that can be obtained show unstable genomes, making them unsuitable for use in regenerative medicine. To address this issue, we propose to employ an approach that previously succeeded in the generation of diseasefree iPSCs for patients with another syndrome of chromosomal instability, Fanconi Anemia. Specifically, we propose to first correct the ATM mutation in cells derived from the skin of an AT patient, restoring the expression of the ATM protein to normal levels ("diseasefree" fibroblasts). We will then reprogram these cells into iPSCs and make sure that they continue to function normally ("disease free" iPSCs). If these experiments are successful, they would warrant additional longterm studies to translate this approach into the clinical setting. For example, cells from the skin, the bone marrow or the cord blood of a child with AT could be "corrected", reprogrammed into iPSCs and then differentiated into other cell types for transplantation. Although we are aware that transplantation of iPSCderived products is not feasible in a clinical setting at the time of this writing, we believe that rapid advances in the stem cell field may facilitate its clinical implementation in the future. We are particularly excited about the most recent development of technologies for the generation of stem cellderived Purkinje neurons and immune system cells, the lineages most affected in AT. Families suffering from AT all over the world have chosen the Johns Hopkins AT Clinic for their care. By bringing the promise of stem cell therapies to this disease, the proposed work will ensure that Maryland continues to be at the center of biomedical research for this disease.

Marta Lipinski, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Parkinson's Disease

Modeling Parkinson's Disease Function of the PARK10 Gene USP24 in Human iPS Cells

Parkinson's Disease (PD) is the second most common agerelated neurodegenerative disease, affecting approximately 1% of all people over the age of 65. Although available PD treatments are able to temporarily decrease disease symptoms, neither a cure nor an effective way to halt or slow PD progression, currently exist. Therefore, there is an urgent need to identify and characterize new genes involved in PD and use them to develop more effective PD treatments. One such gene is USP24, a gene of unknown function which mutations are linked to lateonset PD. USP24 protein levels are also increased in the substantia nigra of subpopulation of idiopathic PD patients. Our data demonstrate that USP24 is involved in the regulation of an intracellular recycling process termed autophagy. Defects in autophagy can lead to accumulation of toxic protein aggregates present in many neurodegenerative diseases and are known to contribute to development of PD. On the other hand, upregulation of autophagy represents an attractive prevention and treatment strategy against PD. Our data suggest that inhibition of USP24 leads to increase in autophagy and may represent a novel treatment against PD. In accordance with the MSCRF goal of developing new treatments for human diseases through human stem cell research, we propose to use normal and PD patient derived induced pluripotent stem cells (iPSCs) as a model to study PD function of USP24. Our findings will also be confirmed in vivo in a rat model of PD. Our goal is to demonstrate that (1) USP24 affects PD by causing defects in autophagy and (2) to determine if inhibition of USP24 can protect against PD neurodegeneration. This information will be necessary for the future use of USP24 as a potential drug target for treatment and prevention of PD and other neurodegenerative diseases.

A-Lien Iu-Chang, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Osteoarthritis

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

When successfully completed, this project will generate significant translational components. Tissue engineering strategies are now being employed, with the goal of improving the quality and longevity of repair tissue and ultimately patient outcome. Because skin is the largest organ in the body, the induce pluripotent stem cells (iPSCs) derived from human primary keratinocytes will be very useful for human therapeutic applications and effectively circumvent the problem of immune rejection. The goal of the project is to advance technology and obtain molecular insights into the reprogramming. This will bring the iPSCs technology closer from bench to bedside. The main challenges of current methods to obtain the iPSCs are the low efficiency, the use of viral vectors, and the tumorigenic potential of the two proto-oncogenes. Another common outcome of factor-mediated reprogramming is incomplete reprogramming that tissue-specific iPSCs harbor „epigenetic memory". Thus, the generated iPSCs vary in quality. Our novel approaches will not only increase efficiency for iPSC induction but also generate iPSCs with high genomic stability. By using DNA repair enzymes and epigenetic regulators, the derived iPSCs should be high proliferative, tolerant to oxidative stress and DNA damage, and highly similar to embryonic stem cells. We do anticipate that successful completion of this exploratory project will permit us to improve iPSCs technology that will enhance and expand their therapeutic use for treatment of damaged tissues. We believe that in the near future the proposed approach to manipulate the cell fate of human iPSCs through alterations in the levels and/or activity of endogenous sirtuins and DNA repair enzymes will be advanced to the translational stage and eventually used for tissue repair. By the end of the project, we believe that the findings will result in our potential to collaborate with local biotechnology companies.

Vasilki Machairaki, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Alzheimer's Disease

Induced Pluripotent Stem Cell Strategies to Model Alzheimer's Disease

This project represents an effort to use stem cell methodologies to develop novel in vitro models for the study of Alzheimer's Disease (AD), an irreversible, progressive brain disease that according to Alzheimer's Association afflicts more than 5 million Americans, or about one in eight over the age of 65. To date, the pathogenetic mechanisms that lead to AD are not well understood. Therefore, there are no treatments currently available that stop or reverse the progression of the disease and no known ways to prevent it. Our application is based on the recent success in our laboratory in reprogramming skin cells from patients with early onset AD into cells similar with embryonic stem cells and differentiating them into human brain cells that they display important disease properties. Here we propose to study AD pathology by a system that allows, for the first time to create, in culture dishes, the hallmark pathology of AD that is amyloid (Aâ) plaques or preplaque formations. With this effort we address a decadeslong aspiration in the field of Alzheimer's disease that is now within reach because of recent major advances in regenerative medicine and materials science. To achieve our goal, we take skin cells from patients with familial Alzheimer's disease caused by mutations in the presenilin gene, as well as from individuals who carry the most common genetic risk factor of Alzheimer's disease, which is the å4 form of apolipoprotein E. Presenilin mutations work to form amyloid when they are present in the primary cells of the brain (neurons). Apolipoprotein å4 helps the formation of amyloid when made in the supporting cells of the brain—for example, astrocytes. In our project, we capitalize on stateoftheart technologies that allow the production of stem cells from skin cells of individuals to manufacture human neurons with presenilin mutations and human astrocytes with apolipoprotein å4. To maximize the chance that our model will generate Aâ plaques or preplaques, we combine these two types of human cells in what we call "cocultures" and we embed these cells in gellike materials. As such, the cells can grow safely in three dimensions and more importantly, can more closely mimic the cellular environment of living organisms. This environment is key in reproducing real brain conditions and in allowing Aâ plaques to form and remain stable. Our project constitutes a pioneering effort to generate models of Aâ plaque formation in the dish based on human cells. In view of several recent negative trials for AD, the field is in dire need for relatively simple, accessible models in which large numbers of antiamyloid but also neuroprotective compounds can be rapidly tested. The availability of simple compelling models of AD as the ones we describe here will substantially increase the level of knowledge of the molecular mechanisms involved in AD pathogenesis, speed up the drug development and may even enable autologous cell transplantation as a clinical therapy in the future.

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

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

Amyotrophic Lateral Sclerosis (ALS), commonly referred to as Lou Gehrig's Disease, is a fatal motor neuron degenerative disease with a median survival time of approximately 36 months. The majority of ALS research is focused on ALS that occurs in families since animal models carrying mutations in ALS genes offer a tool in scientific investigation. However, 90% of ALS cases are sporadic and do not occur in families. Very little is known about what causes sporadic ALS or the way in which it progresses to result in weakness in limbs and ultimately death from breathing failure. Induced pluripotent stem cells (iPSC) provide a powerful tool to study sporadic ALS biology by generating neurons directly linked with patients' medical histories. One fundamental question about ALS is why there is such variability in disease progression; with some patients living less than a year after diagnosis and others living for years or even decades. Prior to the development of iPSC technologies, most studies of ALS conducted in a dish were limited to the culture of neurons from rodent ALS models carrying mutations in the superoxide dismutase (SOD1 gene--representing 1% of all ALS). Studying ALS that only runs in families or is caused from a single gene abnormality is unlikely to represent the variations in disease progression seen in all patients with this disease. The use of iPSC from patients with sporadic and familial ALS allows us, for the first time, to be able to study temporal differences in disease progression using highly manipulable modeling in a disWe propose to characterize the properties of cells from patients with sporadic forms of ALS with differences in the temporal course of disease (slow progressing and fast progressing disease) to better understand the mechanisms contributing to disease progression. We will then compare these patient iPSC properties with patient iPSC carrying familial (SOD1) mutations which are fast progressing (SOD1 A4V mutation) and slow progressing (SOD1 D90A mutation). To improve accuracy in our results, we have an extensive and constantly growing iPSC bank of sporadic and familial cases of ALS, allowing us to create defined groups and enabling greater accuracy in data interpretation. Four groups will be formed: 1) A control group without ALS, 2) ALS slow progression group, 3) ALS typical progression group and 4) ALS rapid progression group. Each group will consist of at least four different lines and contain a combination of sporadic and familial lines, with at least half from sporadic ALS patients. As an Exploratory Grant, the goal of this proposal is to rigorously define the basic characteristics of these iPSC-derived cells and make them available to the stem cell and neurodegeneration communities. We anticipate that beyond this, the innovative approach and subsequent analysis will provide us with a foundation for understanding of sporadic ALS biology, mechanisms contributing to disease progression, and an invaluable tool for future drug screening and biomarker development

David Nauen, M.D
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Epilepsy

Investigating Mechanisms of Epileptogenesis Using Human Induced Pluripotent Stem Cells

Epilepsy, repeated unprovoked seizure, is a prevalent condition that often severely compromises cognitive ability and quality of life, and can be fatal. In most cases the disease is acquired following fever, trauma, or other provoking event. The medial aspect of the brain's temporal lobe is the most common area of epileptic change. Causes of the susceptibility of this region to recurrent seizure are not fully understood, though its specialized neural circuitry and cellular properties are known to contribute. More recently, knowledge of the extent to which new neurons are continually generated from resident stem cells in the hippocampal dentate gyrus within the medial temporal area has greatly increased, and it has been proposed that this neurogenesis contributes to epilepsy. Using rodent models, it has been shown that more dentate gyrus cells are generated following repeated seizure and that many of these new cells become neurons. The impact of these new neurons is not well understood; several lines of evidence indicate that they play a key role in the increased tendency for seizure activity to arise and spread, but some researchers have suggested that they may be compensatory and have an inhibitory effect on the circuit. Assessment of functional changes in the epileptic hippocampus due to newly generated human neurons has been limited, but stem cell technology offers new means of study. Better understanding of the stem cell contribution to epilepsy has the potential to improve patient care and ultimately to allow development of new therapeutic strategies. Our research group is expert in culturing tissue derived from skin in such a way that cells regain stem cell capabilities. These induced pluripotent stem (iPS) cells can then be differentiated into neural progenitor cells. By transplanting these human-derived cells into mouse brain and comparing among animals that experience seizure and those that do not, critical questions can be addressed. The differential survival, migration, and differentiation of these cells in the two conditions can be compared by assessing the tissue microscopically. For epilepsy patients treated surgically, neuropathologists assess the tissue after resection, and the neuropathologist investigators will bring this expertise to assessment of the experimental tissue. To understand what impact these newly born neurons have on the circuit, electrical activity within the regions in questions can be stimulated and recorded for investigation of functional consequences. Our experience with intracellular neuronal recording will be critical here. The abnormal circuit activity in the epileptic temporal lobe is likely to alter patterns of neuronal gene expression. These changes may be limited to the newly generated cells, so assesment at the population level would not detect them. Our laboratory is developing an extremely sensitive method for measuring gene expression changes within individual cells. We will isolate the transplanted human stem cell-derived neurons from seizure-treated and naive mouse brain tissue and characterize alterations in gene expression. This will allow us to develop a list of candidate gene transcripts. Tissue resected from epilepsy patients after surgical resection and not needed for diagnosis can then be used to test for the presence of these altered transcripts, with autopsy brain tissue used as a critical control. Testing human iPS neurons in an animal model, then assessing in human tissue, leverages the strengths of both basic biological research and clinical work with patient tissue. As a result the proposed study offers the prospect of significant medically relevant improvement in our understanding of stem cells in epilepsy.

Tea Soon Park, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Diabetic Retinopathy, Ischemic Retinopathy

Treatment of Diabetic Retinopathy with Human iPSC-Derived Vascular Progenitors

The treatment of vascular disorders by cellular therapy relies on unraveling sources of angiogenic cells in quantities adequate for clinical scale imperatives and capable of longterm functional recovery of the vascular apparatus. Yet, actual candidate sources, e.g., vascular progenitor cells, remain scarce. IPSC may offer new opportunities to produce high levels of specialized progenitors. However, the current conundrum facing their use for regenerative therapy requires the high reprogramming efficiency to be drastically enhanced without the use of viral methods, and to derive functional lineage specific cells. We have developed unique methods to reprogram human CD34+ cord blood and mobilized peripheral blood (PB) cells into pluripotent stem cells using four episomal pluripotency genes (SOX2, OCT4, KLF4, and MYC) reaching unprecedented recovery yields (up to 50%). Also using these nonviral nonintegrated iPSC, we have established vascular differentiation system using embryoid body methods. Here we aim to evaluate in an athymic nude rat model the translational opportunities to treat diabetic retinopathy diseases using vascular progenitors from PBiPSC. First, we will generate PBiPSC from diabetic type 1 and 2 patient blood samples. We have already established CD34+ mobilized PBiPSC lines that provided us the guideline to generate diabetic patient PBiPSC. Once we have established diabetic PBiPSC, we will analyze if their differentiation efficiency toward hematovascular lineages using in vitro and in vivo assays, which we use routinely in our laboratory (including Matrigel tube assay, Matrigel plug assay, DilAcLDL uptake assay). Second, we will establish a diabetic retinopathy disease model in immunodeficient athymic nude rats with our collaborators Drs. Lutty and Bhutto. We will monitor the glucose level daily once we administer streptozotocin to induce diabetes. After confirming acellular blood vessels in the retinas using whole mount retina and immunostaining of retina cryosections, we will introduce PBiPSCderived human vascular progenitors into the eye (vitreous body) or into the circulating blood (tail vein or orbital sinus). We have already established a robust method to detect human cells in the animal eyes using protein luciferase that can be detected using antiluciferase antibody or bioluminescent system in live animals. In order to observe recovery of blood vessel functions, fluorescein angiography will be applied to measure the leakage of blood vessels before and after human cell transplantation. Blood vessel density will be also measured in longterm engraftment assays and revealed by immunohistostainings of the retina layer. This proposal will provide a translational evaluation of the use of human diabetic PB iPSCderived vascular progenitors for cellular therapy of diabetic retinopathy for possible autologous cellular therapy. Our system will offer new foundations in patientspecific regenerative therapies to treat numerous prevalent vascular disorders.

Venu Raman, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $229,920
Disease Target: Inflammatory Bowel Diseases

Manipulating Intestinal Stem Cells to Mitigate the Effects of Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD) is a debilitating disease that tremendously decreases the quality of life and can eventually lead to death. The pathology of IBD is due to alterations to normal cellular constituents of the intestine, which induces a chronic local inflammation that contributes to an autoimmune disease. Healthy cells (epithelial cells) that line the inside of the intestine are constantly exposed opportunistic pathogens and to partially digested dietary food stuffs, which often includes various toxins and mutagenic substances. In general, the cells lining the large intestine are able to manage these insults and remain healthy. A part of this capability is the ability of the epithelial layer to continuously regenerate a healthy epithelium that is derived from so called selfrenewing crypt stem cells. However, on rare occasions, damaged crypt stem cells will survive, divide, and populate the intestinal crypt regions with aberrant cells that subsequently induce IBD. In our research to identify markers that will distinguish healthy stem cells, from damaged one, we have identified an intracellular protein, designed DDX3, which is an essential component of the normal stem cell population. As indicated, the maturation of stem cells ends in terminally differentiated adult cells and the loss of DDX3 expression is a part of this normal healthy process. We now have evidence to indicate that dysregulated overexpression of DDX3 will activate the inappropriate production of inflammatory inducing proteins called cytokines and matrix degrading enzymes, which are major contributing molecules responsible for IBD. In this application, we will systematically characterize, in cellular model systems, how dysregulated DDX3 contributes to an alteration of crypt stem cell in a manner that promotes IBD. We will confirm these findings in patient samples of IBD, specifically ulcerative colitis and Crohn's disease. Importantly, we have patented a drug (RK33) that specifically inhibits the functions of DDX3 and we will test if RK33 can be used as a front line drug for the treatment of IBD. The goal is to revert the cells that induce IBD back to those that give rise to normal terminally differentiated adult cells and in doing so we may be able to provide a treatment that these patients urgently need.

Feyruz Rassool, Ph.D.
University of Maryland, Baltimore
Award Amount: $200,000
Disease Target: Cord Blood Transplant

Efficiently Reprogramed Cells with A MYC Signature Display High Fidelity Repair of DNA Damage

Human induced pluripotent stem cells (hiPSCs) hold great promise in personalized therapeutics for several diseases and in regenerative medicine. While hiPSCs have reprogrammed many cellular responses that are similar to those of human embryonic stem cells (hESCs), many barriers remain to be overcome before they can be used to treat diseases and in regenerative medicine. Among these are two main areas of research to improve hiPSCs: (1) Protocols must be developed for producing hiPSCs in a highly efficient manner. (2) hiPSCs must maintain integrity of the organism so that the genes encoding all the proteins for the propagation of life are faithfully transmitted from generation to generation. Recent reports have suggested that efficient generation of hiPSCs and their genomic integrity are linked in that key DNA repair genes involved in maintaining genomic integrity are required for efficient reprograming of iPSCs. The Zambidis laboratory, expert in stem cell biology at Johns Hopkins University, has recently reported that activation of the master regulatory factor, MYC, and MYC-regulated gene expression module allows for high efficiency reprogramming of cord blood (CB) to CB.iPSCs. Moreover, in collaboration with the Zambidis laboratory, the Rassool laboratory demonstrated that hiPSCs derived with high efficiency also have superior mechanisms for maintaining genomic integrity, including correct repair of DNA compared with hiPSCs derived with low efficiency. Removal of MYC from these cells leads to loss of genomic integrity, suggesting that MYC also regulates genomic integrity. In this study, the Rassool lab in collaboration with the Zambidis laboratory will determine how MYC regulates correct DNA repair in hESCs and hiPSCs. Importantly, they will determine the role of MYC in concert with DNA repair in generating highly efficient hiPSCs. This understanding will allow us to improve the technologies for generating hiPSCs with high efficiency that at the same time have high genomic integrity. The results of this study are critical for the safe use of hiPSCs as a source for cell replacement therapy in humans.

Joseph Stains, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Hutchinson-Gilford Progeria Syndrome

The Role of the Beta-Catenin Signaling Cascade in the Skeletal Phenotype of Hutchinson-Gilford Progeria Syndrome

Hutchinson Gilford Progeria Syndrome (HGPS) has devastating consequences to the affected children. Although rare, this autosomal dominant disease has severe effects—children 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. Patients with HGPS undergo accelerated organ degeneration, and death typically results from coronary artery disease or stroke. Recent therapies have been developed which have been effective at improving the lifespan of these patients, as well as partially restoring skeletal function. However, we propose greater gains in bone quality could be achieved if we simultaneously stimulate new bone formation with a newly developed, highly effective intervention (sclerostin neutralizing antibodies) for improving osteoporotic bone. In this grant, we will use animal models, mesenchymal stem cells that express an HGPS disease causing mutant and induced pluripotent stem cells isolated from patients with HGPS or age matched controls to examine the contribution of a key signaling pathway (canonical âcatenin signaling, which also happens to be the target of the sclerostin neutralizing antibodies) implicated in HGPS to the loss of bone forming cells and the osteoporotic bone found in these patients. This study will test the translational potential of a newly developed therapy for osteoporosis that has great potential to also improve outcomes in this rare disease and if successful will pave the way for a clinical trial.

Jun Wang, Ph.D.
Phycin, LLC.
Award Amount: $100,000
Disease Target: Multiple Diseases

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

Currently the size of the Broader Stem Cell marketplace exceeds $1 billion with 30% annual growth rate. In both the research tool and the cellular therapy space, stem cell culture and type development remain a hotspot. Growth factors are naturally occurring regulatory molecules, which bind to receptors on the cell surface. They stimulate cell and tissue function by influencing cell differentiation. This changes their biochemical activity and cellular growth, regulating their rate of proliferation and activating cellular differentiation. The current mammalian cell and E. coli production platforms are not ideal for recombinant growth factor production. They cannot meet basic research and cellular therapy needs as reflected by the extremely high prices for the commercial growth factors. It is estimated that the growth factors/cytokines account for 25% to 50% of the cost for growth medium. The synthetic biology powered algal platform enables Phycin to produce high quality recombinant proteins economically from both the nuclear and chloroplast genomes in green algae. We propose the development of an algal production platform for the key growth factors used in stem cell biology. With the unique biological features green algae has and the leading algal recombinant protein expression technologies, we anticipate to bring the market prices of recombinant growth factors down to one tenth of their current positions. This will help accelerate the research and commercialization of stem cell biology significantly.

Jiangyang Zhang, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $226,259
Disease Target: Leukodystrophy

Magnetic Resonance Imaging of Myelination by Transplanted Glial Restricted Precursor Cells

In the human brain, the myelin sheath wrapped around axons plays an important role in supporting signal transductions between different brain regions. Diseases that cause damage or loss of the myelin sheath can negatively impact the functions of the brain and lead to severe morbidity and mortality. Recently, it is discovered that transplanted stem cells can potentially repair myelin damage, but there are still many questions to be answered before stem cell based treatments can be introduced to the clinics. Some of these questions include how effectively the transplanted cells repair the damaged myelin and by what mechanisms, and how we can monitor the progress of repair process. To answer these questions, we will develop and validate magnetic resonance imaging techniques and use them to quantitatively examine the extent and time course of myelin repair by a particular type of stem cell, called glial precursor cells, transplanted in a myelin deficient immune deficient mouse model, which has been widely used to study congenital myelin diseases. Because magnetic resonance imaging is noninvasively and readily available in the clinics, the techniques developed in this project can potentially be translated to the clinics.

Zijun Zhang, Ph.D.
MedStar Health Research Institute
Award Amount: $223,779
Disease Target: Bone Defect

Enhancing the Incorporation of Bone Allograft with Circulating Mesenchymal Stem Cells

Segmental bone loss caused by either trauma or resection of bone tumor is usually repaired by transplantation of bone allograft, a cadaveric bone vigorously processed and sterilized at tissue banks. This type of reconstructive surgery restores the continuity and mechanical strength of the damaged bone and is commonly performed. Cadaveric bone allograft has fewer supply constraints than bone autograft that is taken from other parts of the patients. The natural contour and inner structure of allograft are superior to synthetic bone substitute materials. The long-term clinical outcome of bone allograft, however, is not satisfactory. Complications of bone allograft, such as fracture, infection and non-union with host bone, lead to a 10-year failure rate as high as 65%. The acellular nature of bone allograft is generally believed that impairs the healing of bone allograft. Bone allograft is stored in deep freeze or frozen-dry, which effectively eliminates viable cellular elements in bone. While this procedure is necessary for the recipients' safety and reducing immune reaction of allograft, it leaves bone allograft virtually a dead bone. The persistence of non-viable allograft causes delayed union or non-union with host bone, and increases the risks of infection and fracture. The long term goal of this project is to revitalize bone allograft and improve its incorporation with host bone through enhanced blood vessel formation and bone regeneration. In fracture healing, an increased SDF-1 (stromal cell derived factor-1) concentration at fracture site is critical. SDF-1 is a protein produced by cells in blood vessels and can recruit CXCR4-expressing mesenchymal stem cells (MSCs) to the fracture site for bone regeneration. A SDF-1 gradient that attracts MSCs, however, is absent in acellular allograft. We hypothesize that, by coating allograft with SDF-1 and intravenous administration of CXCR4-expressing MSCs, the allograft not only regains but also is enhanced the capability of recruiting MSCs in circulation. As a result, the allograft is quickly revitalized and improved incorporation with host bone. Previously, we isolated MSCs from bone marrow based on a cell surface marker—CD271. CD271+MSCs repaired bone defects, when implanted into mice. Furthermore, we demonstrated that, after injected intravenously, CD271+MSCs travelled to and accumulated at the femoral fracture site in mice. In tissue culture, these CD271+MSCs responded to and moved toward SDF-1 containing medium. Being able to form bone, travel to the fracture site, and migrate toward a SDF-1 gradient, CD271+MSCs fulfill the requirements for enhancing allograft revitalization and incorporation with host bone. In this proposed study, we will investigate 1) the methodology of coating allograft with SDF-1. The coating conditions, including SDF-1 concentrations, will be adjusted to produce SDF-1/allograft that is capable of attracting CD271+MSCs for an extended period. 2) SDF-1/allograft and allograft alone, as controls, will be implanted into 5-mm femoral defects in athymic mice. CD271+MSCs isolated from human bone marrow will be intravenously injected at 1, 2 and 3 weeks post allograft implantation, with injection of saline as controls. Combinations of implantation of SDF-1/allograft or allograft alone and with or without intravenous injection of CD271+MSCs, will be quantitatively assessed. The effectiveness of circulating MSCs and SDF-1 coating on allograft revitalization and incorporation with the host bone will be comprehensively analyzed for an optimized strategy to enhance angiogenesis and osteogenesis in the bone allograft. This proposed study is highly relevant to MSCRF mission "of achieving human stem cell therapies to repair or replace missing, damaged, or diseased cells, tissues and organs". Clinical translation of the strategy of using circulating MSCs to enhance the revitalization and incorporation of bone allograft has the potential to improve the outcome of bone allograft and broaden allograft applications.

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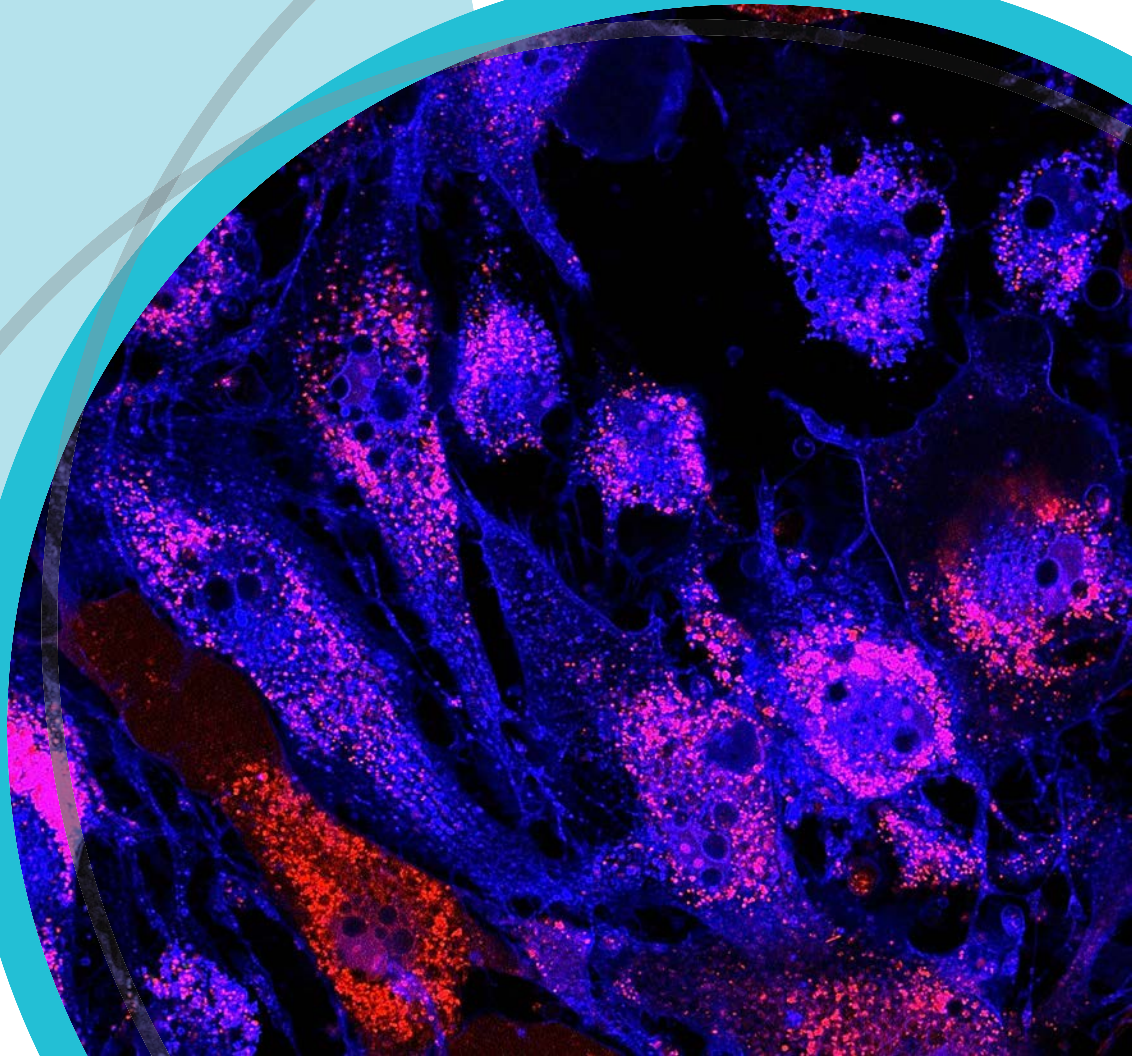
2014
Post-Doctoral Fellowship Grant Awards:



Tong Ma, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Guo-li Ming, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Schizophrenia

Investigating the Role of a Mental Disorder Risk Gene in the Development of Human GABAergic Neurons

Schizophrenia is a chronic neuropsychiatric disorder characterized by aberrant perceptions, cognitive processes, and behavior, which affects 1% of the world's population [1]. While the etiology is poorly understood, a number of postmortem studies have provided consistent evidence that a defect in GABAergic transmission plays a role in both schizophrenia and major depressive disorders [2, 3]. It has been postulated that such a disturbance may be related to a perturbation of early development, one that may result in a disturbance of cell migration, the formation of normal lamination and functionally intact synaptic development [4]. How risk genes for schizophrenia affect the development of human GABAergic interneurons is completely unknown. Disrupted-in-schizophrenia 1 (DISC1) has been identified as a risk gene that associates with schizophrenia and major depressive disorders in several patient cohorts [4, 5]. In an American family (Pedigree H), many members carry a four-nucleotide deletion between exon 12 and intron 12 in the DISC1 gene, the majority of whom have been diagnosed with schizophrenia, schizoaffective disorders and major depression. Using induced pluripotent stem cells (iPSCs) generated from Pedigree H and cerebral organoids derived from these iPSCs [6], I will investigate how a disease-relevant DSIC1 mutation regulates the development and integration of human GABAergic interneurons with three specific aims:

- **Aim 1**: To characterize the role of DISC1 in differentiation of human GABAergic interneurons
- **Aim 2**: To assess the structural development of human GABAergic interneurons with a DISC1 mutation
- **Aim 3**: To measure the impact of DISC1 mutation on the functional properties of GABAergic interneurons derived from human iPSCs.

Our proposed research project tests the hypothesis that mutations in the mental disorder risk gene, DISC1, impair the structural and functional integrity of human cortical interneurons. Data generated from the successful completion of these experiments will provide important new insights into how the GABA transmission system may be altered in neuropsychiatric disease.

Manoj Kumar, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Ted Dawson, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Parkinson's Disease

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

Parkinson's Disease (PD) is one of the most common neurodegenerative disorders in which there is selective and progressive loss of dopaminergic (DA) neurons. Mutation in specific genes including DJ-1, PARKIN, PINK1, LRRK2 and α-Synuclein has been shown to cause PD (1). Parkin is one of the genes where more than 100 mutations have been reported to cause PD due to its loss of function (2). Parkin plays a major role in PD and its inactivation is one of the major causes of both sporadic and familial form f PD (3). Latest discoveries indicate that parkin is a key regulator of mitochondrial homeostasis including organelle biogenesis, fusion/fission and clearance (4). Recent studies in drosophila confirm that the absence of parkin leads to mitochondrial impairment similar to autosomal recessive PD in humans (5). The mechanism by which parkin regulates mitochondrial function and integrity are still unknown. This may be due to the fact that current working models based on mice, flies or cell lines do not fully recapitulate the cellular and molecular events occurring in human DA neurons in PD. Generating human DA(hDA) neurons from human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) has the potential to provide a suitable working model for PD. In this proposal, using well-established protocols we will create a parkin gene conditional knockout (cKO) hESCs and hiPSCs isogenic lines (parkin cKO isogenic lines). Inactivating the function of parkin in hDA neurons will be used to study its role in PD neurodegeneration. In a parallel study genome scale screening for rescue of mitophagy will be investigated on hDA neurons by CRISPR-cas9 knockout technology. Deep sequencing analysis on surviving cell after CRISPR/cas9 KO will be analyzed for essential genes whose loss of function contributes to reduced mitophagy. The role candidate genes after screening will be studied on the parkin cKO isogenic lines.

- **Specific Aim 1**: Generation of isogenic human parkin PD pluripotent stem cell lines.
- **Specific Aim 2**: Characterization of mitochondrial function, deficits and biogenesis in the human parkin PD pluripotent stem cell lines.
- **Specific Aim 3**: To conduct a CRISPR/Cas9 KO genome screen for rescue of mitophagy in hDA neurons.

Sang Hoon Kim, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Hongjun Song, Ph.D.
Award Amount: $110,000
Disease Target: Double Cortex Syndrome

Modeling and Characterization of Double Cortex Syndrome Using iPSCs and Cerebral Organoids

Double Cortex Syndrome (DCS), also known as subcortical band heterotopia (SBH), is a congenital brain disorder that arises from aberrant neuronal migration during development of the cortex. It has the appearance of an extra layer of neurons that are under the normal gray matter of brain cortex. Double cortex syndrome patients often have epilepsy and cognitive impairments (1). A mutation in the X-linked gene doublecortin, DCX, is the primary cause of the disease in female patients. However, the etiology and the molecular mechanisms of double cortex syndrome in male patients are poorly understood. For this study I will characterize induced pluripotent stem cells (iPSCs) derived from patients with double cortex syndrome to investigate the biological pathways and cellular phenotypes that may underlie this disease. We have so far generated iPSCs from skin fibroblasts of male double cortex syndrome patients and from unaffected parents (Fig. 1). Recently, a fascinating new method was developed to generate cerebral organoids, three dimensional brain-like structures from human pluripotent stem cells in vitro (2). This "minibrain" recapitulates a surprising number of features of human embryonic brain development, and therefore provides a new phase in modeling human disease and an optimal in vitro system to examine phenotypes related to neuronal development, migration, and structural organization. Using multiple approaches, including electrophysiology, immunostaining, and imaging, I will receive training in the human stem cell field and characterize potential cellular and molecular mechanisms of double cortex syndrome with the following two specific aims:

Aim 1: To characterize neural differentiation of iPSCs from patients with double cortex syndrome in vitro and after transplantation;

Aim 2: To investigate potential cellular mechanisms of double cortex syndrome using cerebral organoids from patient-derived iPSCs.

We already have all the reagents in hand and already have some initial success. I expect that cerebral organoids from double cortex syndrome patients may have neurodevelopmental defects such as presence of heterotopia and abnormal neuronal migration. It is also possible that neurons in cerebral organoids derived from patient iPSCs might show abnormal excitability, which may correlate with seizure-related activity. One current issue with the organiod system is that only few cortical layers are formed, due to cell death in the core when its size get too big. We have been working with bioengineers at Hopkins to add nanochannels into the organiods to facilitate the transfer of nutrients into the core.

Hui Lin, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Samuel Yiu, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Dry Eye Syndrome; Lacrimal Gland Dysfunction

Bioengineering a Lacrimal Gland Using Human Lacrimal Stem Cells

The health and integrity of the ocular surface depends greatly on the stability of the tear film that covers the anterior surface of the eye. The tear film has three basic layers - the outer thin lipid layer secreted by the meibomian glands, the middle bulk of aqueous layer secreted by the lacrimal gland (LG) and the inner mucous layer secreted by the ocular surface epithelial cells. LG dysfunction results in aqueous deficient dry eye, which can lead to eye infections, impaired wound healing, scarring of the cornea and blindness. With the estimated global prevalence of dry eye ranging from 5% to over 35% at various ages, a huge number of adults suffer from dry eye in the world. The present treatment of dry eye involves instillation of ocular surface lubricants which may not help severe dry eye patients with irreversible damage of lacrimal secretion function. Regeneration of the lacrimal gland using lacrimal stem/progenitor cells may potentially restore LG function and therefore, improve the ocular surface health. LG contains at least 3 types of cells, the acinar cells, the duct cells and the myoepithelium. Lacrimal acinar cells are the majority cell type with significant secretory function. While several methods have been developed to maintain LG cells of multiple species in vitro, preservation of the acinar cell phenotype and secretory function has proven to be difficult(1). The inconvenience of expansion and maintaining ex vivo restricts the direct usage of acinar cells in LG regeneration (2, 3). In contrast, evidences from regenerative medicine suggest that tissue specific stem / progenitor cells can provide a promising cell resource (4). Several studies on salivary glands, submandibular glands, pancreas have demonstrated that stem/progenitor cells can be isolated, expanded and differentiated into both acinar and ductal cells in vitro (4-6). These encouraging results have raised hope for fabrication of a functionally competent gland system using lacrimal stem cells. K14+positive staining cells were ductal cells in uninjured rabbit LG, and the duct ligation can induce significant LG regeneration in rabbit.We hypothesized the origin of LG stem/progenitor cells from the duct in rabbits and have established a novel ex-vivo culture system for these cells. While the understanding of stem cells in animal models has increased, there is a paucity of data concerning the human LG. To the best of our knowledge, only one study has reported the presence of putative stem cells in human LG. Evidence from Tiwari et al's study suggested that a heterogeneous population of cells containing a relatively rare stem- cell like subpopulation can be isolated from native human LG(13). However, the stem cell marker positive cells decreased dramatically during culture period in their culture system. Their data provided evidence for the existence of stem cells in the native human LG tissue which can be recruited to salvage the function of the damaged gland. They also showed that the human LG cells can be cultured in-vitro with retained secretory function. However, the purification and characterization of the human lacrimal stem/progenitor cells is still lacking. Our present proposal will address these questions, aiming to provide the suitable cell sources to bioengineer a lacrimal gland for clinical transplantation(14). Our preliminary studies on rabbit LG stem cell culture and decellularized scaffold as bioengineering materials will provide us with a solid platform to construct the human LG. The distinguished clinical experience of our team members in ocular surface diseases will also promote the application of lacrimal stem cell based therapy in animal models and then human beings in future.

Lipeng Tian, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Yoon-Young Jang, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Organ Transplantation

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

Alcohol-induced liver disease (ALD) is a major cause of illness and death in the United States [1,2]. This includes fatty liver, alcoholic hepatitis, and chronic hepatitis with fibrosis/cirrhosis [2,3]. Currently there is no effective treatment for curing or preventing progression of ALD [4]. Liver transplantation can be done for only a limited number of the end stage liver failure patients depending on organ availability [5]. Novel strategies to prevent or treat ALD are urgently needed. Compared to another major liver disease, viral hepatitis, which has been extensively studied and consequently prevention/therapies are currently available, ALD is under-studied and even how alcohol damages the liver is not clearly understood. This is mainly because there is no human-relevant model system to study the effect of alcohol on human liver tissue. Animal models often inaccurately represent human pathophysiology. There are no human cell lines established from ALD patients unlike neoplastic diseases where cell lines are frequently available. The emerging induced pluripotent stem cell (iPSC) technology provides an alternative for generating functional, renewable and human relevant cell sources for disease modeling using patient tissues as we have recently shown with an inherited liver disease [6-8]. In addition, we have generated patient iPSCs from multiple liver diseases including ALD [9-13]. Taking advantage of this iPSC resource and hepatic differentiation technologies established in our laboratory, I propose to develop an in vitro model of ALD. Chronic consumption of alcohol results in the secretion of pro-inflammatory cytokines, oxidative stress, and acetaldehyde toxicity [2]. Since these factors cause pathologic phenotypes of ALD (i.e. fatty change, inflammation, apoptosis and fibrosis), we plan to use a multiparametric approach to capture/assess the cellular pathology for disease modeling. Parameters for both basic hepatotoxicity and pathological effects of alcohol on iPSC derived multi-stage hepatic cells will be evaluated. The most reliable condition will be selected for disease modeling and then further optimized for effective drug iscovery. This project will test the hypothesis that iPSCs will provide effective tools for modeling ALD in vitro, which could provide a molecular understanding of the disease process as well as enable the discovery of novel therapies for ALD patients without other therapeutic options.

Aim 1: To determine cytotoxicity of alcohol and its metabolite on human iPSC-derived multistage hepatic cells by evaluating their viability, proliferation, differentiation, and function.

Aim 2: To recapitulate key disease phenotypes of ALD in a dish by determining the effects of alcohol on fatty change, inflammation and collagen accumulation in iPSC-derived hepatocytes.

These aims, although independent from each other, are targeted for a common objective of establishing a human ALD model. Regardless of the experimental outcome, we will be able to obtain useful information about stem cells, alcohol and liver disease, to be used for regenerative medicine.

Anna Jablonska, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Piotr Walczak, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Stroke

Genetic Engineering of Glial Progenitor Cells for Improved Intraarterial Targeting In Stroke

Stroke is a leading cause of death and chronic disability in adults. It burdens public health systems and, due to aging of Western societies, is expected to cause increasing costs for hospitalization and rehabilitation. The only established treatment for stroke is thrombolysis. However, this therapy is only available within a narrow 4.5 hour time window - currently leaving more than 90% of patients untreated. Consequently, there is a strong demand for novel therapeutic approaches that target subacute and chronic phases after cerebral ischemia. One of the most attractive strategies to enhance post-stroke recovery is stem cell therapy. Various types of stem cells are intensely investigated as candidates for treatment, however based on our preliminary data we postulate that progenitors of oligodendrocytes appear as the most promising. We have observed in rodent models that subcortical stroke is related to relatively good preservation of neurofilaments, but dramatic and rapid degradation of myelin within the stroke area (Fig.1). This shows that oligodendrocytes are more vulnerable in comparison to axons. Thus restoration of myelin is an excellent therapeutic target and the replacement of oligodendrocytes is a viable option to support recovery. We have already shown positive effect following transplantation of GRPs in rat model of transverse myelitis (Walczak et al., 2011). We also observed extraordinary therapeutic effect in dysmeylinating shiverer mice. Neonatal transplantation of GRPs resulted in an excellent and widespread engraftment of GRPs, and moreover they practically rescued normal life-span of shiverer mice (extension of lifespan from ca. 200 to over 400 days). Overall, the GRPs are highly therapeutic cells and they may play a role in recovery from stroke. Consequently, we intend to further improve this methodology and utilize novel mRNA-based technique which according to our preliminary evidence has much more desirable features for our needs including: rapid onset and short lasting expression, very high transfection efficiency and very good safety profile. We have shown that in contrast to DNA-based methods, mRNA as shown by eGFP mRNA transfections very efficient in primary cells (Fig. 3), it is considered one of the safest gene delivery methods for clinical use since there is no risk of integration into the genome and expression is always silenced following degradation of mRNA. Thus we propose mRNA transfection as a strategy to transiently express both subunits of VLA-4 molecule and use this strategy to develop clinically applicable method of cell trafficking. Functionality of engineered cells will be evaluated using in vitro microfluidic assays and intravital microscopy in rat model of stroke.

Ian Martin, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Ted Dawson, M.D., Ph.D.
Award Amount: $110,000
Disease Target: Parkinson's Disease

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

LRRK2 mutations are a common cause of familial Parkinson's disease (PD) and increase susceptibility to developing sporadic PD (Cookson, 2010). Importantly, cell and animal models have linked LRRK2 toxicity to its kinase activity which is increased by the common G2019S LRRK2 mutation (Smith et al., 2006; West et al., 2007). Consistent with this, inhibition of LRRK2 kinase activity by potent and specific LRRK2 kinase inhibitors can prevent LRRK2 toxicity in vivo (Deng et al., 2011; Ramsden et al., 2011). I observed that the protein synthesis machinery is a key target for LRRK2 kinase activity and that G2019S LRRK2 increases ribosomal substrate phosphorylation and stimulates cap-dependent and cap-independent translation which plays a pivotal role in LRRK2 toxicity (Martin et al., submitted). Prior reports show that manipulation of core translation components do not result in uniform effects on all cellular transcripts (Gkogkas et al., 2013; Thoreen et al., 2012). Therefore, I hypothesize that LRRK2 regulates translation in a gene-specific manner and that altered gene-specific translation by pathogenic LRRK2 underlies cellular toxicity leading to PD development. My goal for this project is to perform translational profiling of wild type and G2019S LRRK2 human dopamine neurons acutely following the administration of established LRRK2 kinase inhibitors in order to identify direct translational targets of LRRK2 kinase activity in healthy and disease-prone neurons. Quantifying proteome dynamics in this manner should enable us to distinguish direct targets of translational regulation from steady-state proteomic alterations in mutant PD patient lines which may arise through secondary and other indirect effects on gene expression. This promises to greatly advance our understanding of PD pathogenesis. It will also be informative to compare altered translational profiles from wild type and G2019S LRRK2 neurons following kinase inhibition to determine the degree of similarity in affected transcripts. Using human dopamine neurons promotes iscover of translational targets of LRRK2 relevant to the human disease process.

Aim 1: Effect of LRRK2 kinase inhibitors LRRK2-IN-1 and CZC-25146 on global protein synthesis rates in wild type and pathogenic G2019S LRRK2 human dopamine neurons

Aim 2: Quantitative proteomic profiling of wild type and G2019S LRRK2 human dopamine neurons following LRRK2 kinase inhibitor treatment

Raju Khatri, Ph.D.
University of Maryland, Baltimore
Mentor: Michal Zalzman, Ph.D.
Award Amount: $110,000
Disease Target: Osteoporosis; Stem Cell Therapy

Increasing the Replicative Lifespan and Quality of Adult Mesenchymal Stem Cells

The clinical application of adult stem cells has been limited due to their rapid decline in both differentiation and expansion potential. This decline is associated with physiological aging1-4, which hinders reproducibility and feasibility of treatments. Telomeres serve as a biological countdown clock, restricting cell proliferation and differentiation, ultimately leading to senescence and apoptosis6. Conventionally, telomere length has been considered to be maintained by telomerase7,8, but we have shown that a transient expression of Zscan49-11 can restore length of telomeres in mouse embryonic stem (ES) cells independently of telomerase. We propose that transient activation of ZSCAN4 will increase telomere length and therby the multipotency of human mesenchymal stem cells (hMSCs).

Aim 1: To increase MSCs replicative lifespan and potency.

Aim 2: To study the ZSCAN4 mechanism and its effect on MSCs genome stability.

Our preliminary data demonstrates our ability to isolate, propagate and maintain MSCs with multipotency. Activation of ZSCAN4 by transient transfection rejuvenates MSCs and leads to a dramatic increase in telomere length. Moreover, ZSCAN4 is localized on telomeres in human MSCs (Fig.3). In this proposal, we will expand upon these findings to establish ZSCAN4 as a critical component of SCs rejuvenation.

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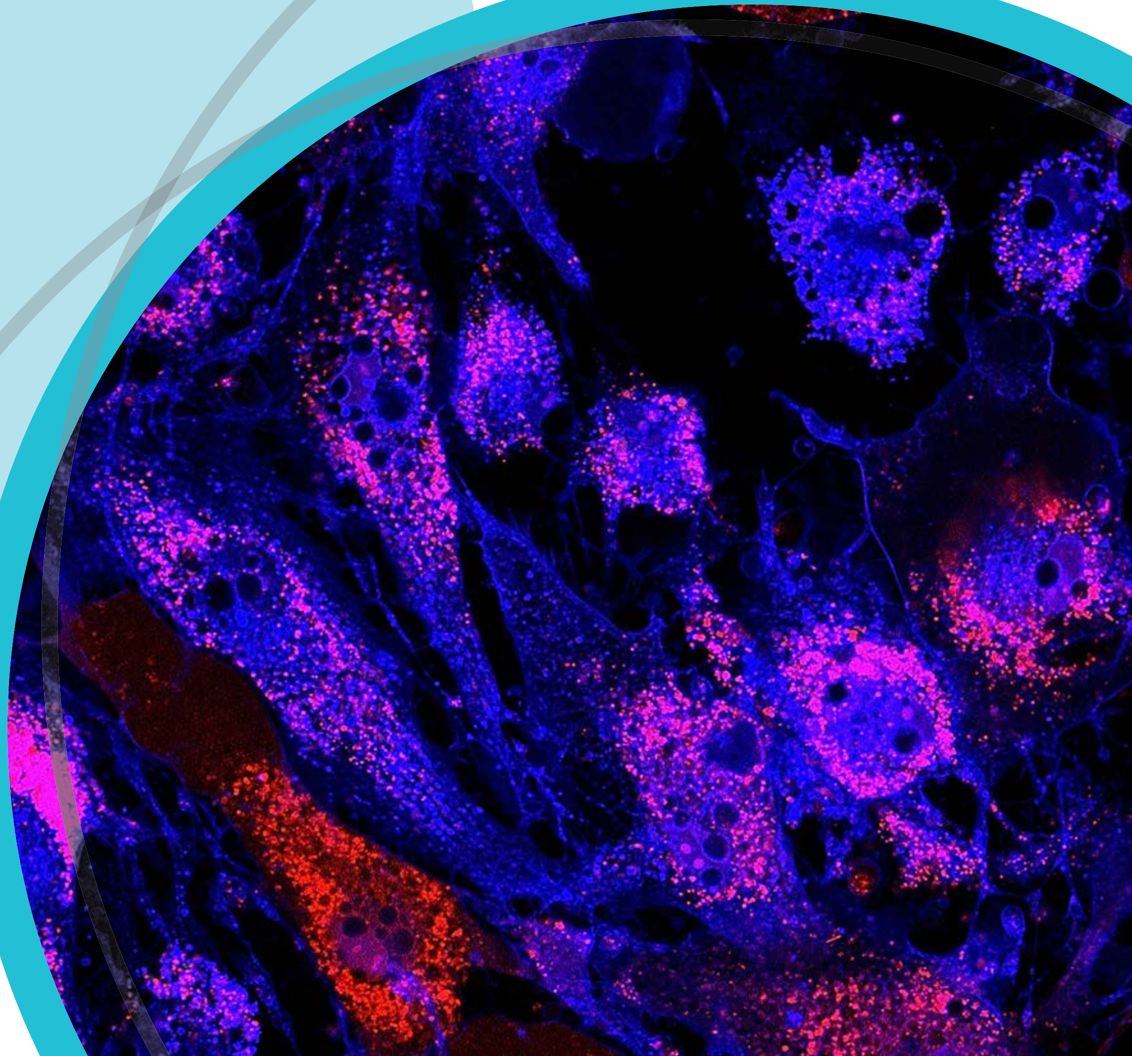
MSCRF Grants Completed:

{Calendar Year 2014}



John Laterra, M.D., Ph.D.
Hugo W. Mosser Research Institute at Kennedy Krieger
2011 Investigator Initiated Award Budget: $690,000
Disease Target: Multiple

Regulation of Neural and Neoplastic Stem Cells by Kruppel-like Transcription Factors

We establish for the first time a genome-wide map of KLF9-regulated targets in human glioblastoma stem-like cells, and show that KLF9 functions as a transcriptional repressor and through its transcriptional repressive functions regulates multiple signaling pathways involved in neoplastic stem cell regulation.

A first ever genome-wide analysis of KLF9 targets was performed by combining gene expression profiling using RNA-seq and mapping of transcription factor binding sites using ChIP-seq in GBM neurospheres with ectopic KLF9 induction. Over 150 million reads were generated from ChIP-enriched DNA libraries and input controls derived from two biological replicates. Using CisGenome, 31,261 KLF9 binding peaks were called at a false discovery rate (FDR) of 1%. The majority of KLF9 binding sites localized to intergenic regions (54.0%) and introns (36%), and binding sites localized less frequently to 5'- untranslated regions (7%), exons (11%) and 3'-untranslated regions (1%) (Figure 2E). KLF9-binding sites were found to be highly enriched (66% of peaks) around transcription start sites consistent with a transcriptional regulatory function. De novo motif discovery analysis identified 5'-G/A G/T GGG C/T G G/T GGCN-3' as the most enriched KLF9 binding motif.

By combining KLF9 ChIP-Seq and RNA-Seq datasets we were able to determine that KLF9 functions primarily as a transcriptional repressor in GBM stem cells and ~1,849 genes directly downregulated through KLF9 promoter site binding were identified. Gene function annotation and Ingenuity Pathway Enrichment Analysis of these genomic KLF9 targets revealed that KLF9 regulates multiple pathways important to oncogenesis and cell fate determination including CXCR4 signaling, integrin signaling and notch signaling, stem cell pluripotency signaling, axonal guidance signaling, CREB signaling, and semaphorin signaling. Integrin signaling, which modulates multiple cellular processes including cell adhesion and migration, tumor cell invasion and cell stemness, was one of the top ranked and validated pathways enriched in KLF9 downregulated targets. ITGA6, encoding integrin α6 receptor subunit and other downstream components of the integrin signaling pathway, such as ARPC1B, CAPN5, GIT1 and MYLK, were validated to be substantially downregulated by KLF9.

The results outlined above offered us the opportunity to begin to identify and functionally dissect specific KLF9-repressed gene targets that regulate GBM cell stemness and tumor propagating potential. One theme emerging from our findings is that KLF9 regulates GBM cell stemness by inhibiting the expression of multiple genes that regulate cell-cell and cell-matrix interactions and integrin - mediated mechanisms. We found that downregulation of ITAG6 by KLF9 differentiates GBM stem cells as evidenced by loss of self-renewal capacity, downregulation of molecular markers and drivers of cell stemness, and inhibited capacity to generate orthotopic tumor xenografts. We are also finding that expression of another KLF9 target, the transmembraneous integrin-binding protein CD151 (tetraspanin), is required for maintaining GBM cell stemness and self-renewal capacity.

In summary, we have identified human gene targets directly regulated by KLF9 on a genome-wide scale in GBM-derived stem cell models. We found that KLF9 inhibits GBM cell stemness and functions predominantly as a transcriptional repressor within this cellular context. KLF9 is shown to regulate multiple signaling pathways including those involved in oncogenesis, stem cell regulation, neuronal cell signaling, and integrin signaling. KLF9 is shown to inhibit GBM cell stemness and tumorigenicity and that these effects result from downregulatign multiple gene targets that regulate cell adhesive and integrin functions. These findings enhance our understanding of the transcriptional networks underlying CSC maintenance and differentiation, and provide new regulatory mechanisms applicable to cancer therapeutics.

Tami Kingsbury, Ph.D.
University of Maryland, Baltimore
2012 Exploratory Award Budget: $230,000
Disease Target: Transplant Relevant Diseases

MicroRNAs and Control of Quiescence and Pluripotency

Although stem cells hold great promise for regenerative medicine, their clinical application is limited by our inability to ex vivo expand stem cells without changing their cell properties. Low levels of cellular reactive oxygen species (ROS) levels are a hallmark of quiescent stem cells and have been implicated in maintaining stem cell integrity and pluripotency (the ability to give rise to multiple types of cells). Hematopoietic stem cells (HSCs) have the intrinsic ability to self renew and to give rise to cells which can differentiate into all blood cell lineages. Hematopoietic stem-progenitor cells (HSPCs) are used clinically for bone marrow transplants, but their low abundance (~1% of bone marrow cells) limits their clinical potential. Currently, a single bone marrow donor's sample provides sufficient HSPCs for only a single transplant recipient. The ability to transplant higher numbers of HSPCs per patient would also reduce the morbidity and mortality associated with transplants, by increasing engraftment efficiency and reducing the time required to regenerate a functional hematopoietic system. Methods to drive HSPC self-renewal ex vivo are urgently needed and could have profound impact on the availability of these critical cells for patients in need of transplants. We decided to take advantage of microRNAs to discover potential new methods to promote HSPC expansion ex vivo. MicroRNAs are key regulators of gene expression that can coordinate complex biological responses to developmental and environmental cues by targeting multiple genes simultaneously. We proposed to take advantage of our recently developed functional microRNA screening platform to identify microRNAs that can modulate hematopoietic stem/progenitor ROS levels. During preliminary experiments, however, we discovered that the commercially available microRNA library could not efficiently drive microRNA expression in hematopoietic cells. We therefore, conducted a functional screen in a human cell line HEK293, to identify microRNAs that altered the ability of cells to grow and/or survive in response to elevated ROS. This screen has the potential to identify microRNAs that not only modulate cellular ROS levels, but also cellular responses to increased ROS.

(Aim 1) A library of approximately 600 microRNAs was screened in human HEK293 embryonic kidney cells in the presence or absence of hydrogen peroxide-induced ROS. By comparing control versus hydrogen peroxide-treated cells in parallel, we were able to distinguish microRNAs with general growth-inhibitory activity from those microRNAs that altered growth only under conditions of elevated ROS. Analysis of two independent screens identified 85 microRNAs selectively lost from the cell population during culture in the presence of hydrogen peroxide and 5 microRNAs selectively enriched. MicroRNAs lost during the screen presumably increase cellular ROS or the sensitivity of cells to ROS damage. In contrast, microRNAs enriched during the screen may reduce ROS levels, ROS-mediated damage or cellular responses to ROS-mediated damage. Both classes of microRNAs have the potential to identify critical signaling pathways to target in order to increase stem cell resistance to elevated ROS encountered during ex vivo expansion. We therefore constructed a lentiviral sub-library expressing the microRNA candidates under the EF-1 promoter. This promoter enables efficient microRNA expression in hematopoietic cells. Verification of microRNA expression and the consequences enforced expression of individual microRNAs on growth and differentiation was initially assayed in the CD34+ human erythroleukemia cell line TF-1. From this analysis we identified two microRNAs that modulated TF-1 cell growth and blocked erythropoietin-induced differentiation.

(Aim 2) Enforced expression of each of the top two candidate microRNAs in HSPCs resulted in an increased percentage of HSPCs expressing the CD34+ stem cell marker during a time course of 10 days in culture. Using luciferase 3'UTR-reporter gene analysis, we have demonstrated the ability of these microRNAs to directly target several genes linked to signaling pathways potentially involved in the regulation of stem cell proliferation or differentiation in diverse tissues. Future in vivo experiments will determine whether these microRNAs expand functional HSPCs and determine the key gene targets by which they exert their effect. We believe that these microRNAs will allow us to discover key factors and pathways to target in order to develop methods to enhance HSPC expansion ex vivo. Ultimately, we hope that our results in HSPCs will serve as a paradigm for the regulation of stem cells derived from diverse tissues and the development of methods to generate cells needed for clinical applications.

Chulan Kwon, Ph.D.
Johns Hopkins University
2012 Exploratory Award Budget: $230,000
Disease Target: Heart Diseease

Membrane Notch Control of Human Cardiovascular Progenitors

Heart disease is the number one killer of both men and women. It is responsible for the death of nearly one in every four people in the United States. The human heart lacks the ability to repair itself after injury b/c cardiac muscle cells lose regenerative capacity after birth. Consequently, each year about 1 million Americans, who survived a heart attack, are left with failing hearts that are typically irreversible. Over 5 million Americans suffer from heart failure and more than 250,000 people die annually. However, no treatment can cure a failing heart. For these reasons, current cardiac regenerative research has focused on developing foreign cell sources to repair damaged hearts. Cardiac progenitor cells (CPCs) are the building blocks used to generate the heart during fetal development. They can be derived from human pluripotent stem cells (PSCs) and have the unique capability to expand and form mature heart muscle cells in the adult heart.

With this capability, CPCs have tremendous regenerative potential to cure heart disease. However, crucial roadblocks must be overcome for their clinical application. Current methods for obtaining CPCs from human PSCs are not simple and rapid enough for timely treatment of heart attack.

In addition, there is no effective method available for their maintenance and delivery. During the funding period, we discovered potent factors, including Numb family proteins, noncaonical Notch, and fibronectin, and their mechanisms that control CPC induction and maintenance to overcome the current roadblocks for clinical application of PSC-derived CPCs.

These findings will greatly advance our knowledge of human CPC biology and allow us to develop novel strategies to fast-track production and long-term maintenance of human PSC-derived CPCs, which will accelerate PSC-based interventions of cardiac repair.

Sivaprakash Ramalingam, Ph.D.
Johns Hopkins School of Public Health
2012 Exploratory Award Budget: $230,000
Disease Target: Sickle Cell

Functional Correction of hiPSCs with Homozygous Sickle Cell Disease Mutation Using Engineered ZFNs/TALENs

Gene editing nucleases-zinc finger nucleases (ZFNs), transcription activator- like effector nucleases (TALENs) and CRISPR/Cas9 - have conferred molecular biologists with the ability to site-specifically and permanently alter plant and mammalian genomes, including the human genome via homology-directed repair (HDR) of a targeted genomic double-strand break (DSB). The main focus of our project was on in situ editing of the HBB gene mutation in patient-specific sickle cell disease (SCD) hiPSCs' using engineered TALENs. We have successfully achieved monoallele correction of the SCD mutation in patient specific SCD hiPSCs.

Genetic correction of HBB mutation in patient-specific SCD hiPSCs using TALENs: TNC1 hiPSCs (a patient-derived line carrying a homozygous SCD mutation) were transfected with HBB-specific TALENs and the donor plasmid, which contained both GFP and puromycin gene cassette flanked by loxP sites and left and right homology arms with T to A replacement in the βS allele; this resulted in monoallele correction of HBBS locus by the HBBA gene via HR. SCD-corrected hiPSCs were transiently transfected with Cre recombinase gene to remove the selection cassette from the monoallele HBB-corrected SCD hiPSCs. After negative selection for GFP by FACS, GFP- cells were distributed as 1 cell/well into 96-well plates and grown to obtain single cell colonies. The heterozygous βS/βA SCD hiPSC colonies were characterized for expression of pluripotency markers pre- and post-Cre treatment.

Erythroid differentiation of monoallele HBB-corrected βS/βA SCD hiPSC lines: Since the undifferentiated hiPSCs do not express the HBB gene, we pursued in vitro hematopoietic differentiation of heterozygous βS/βA SCD hiPSC lines to generate HBB-expressing erythroid cells. Two-step serum-free embryoid body (EB)-mediated hematopoietic differentiation procedure was used to differentiate the hiPSC lines. In step 1, cystic embryoid bodies (EBs) were efficiently formed from hiPSC lines in the presence of cytokines. In step 2, we generated the erythroid cells from the EBs in the presence of cytokines. Gimsa staining confirmed that most of the differentiated cells are erythroblasts.

HBB expression in monoallele HBB-corrected βS/βA SCD hiPSC-derived erythrocytes: In order to measure the total expression of the HBB gene (including βS and corrected βA allele) and fetal type (HBG) genes, we performed quantitative RT-PCR in SCD hiPSCs, C2, Cre26, Cre33 hiPSC lines at erythroblast stage [Fig. 5(iii)]. The level of HBG expression in erythroblasts derived from βS/βS SCD hiPSCs, βS/βA SCD-corrected hiPSCs (C2) and Cre-treated βS/βA SCD-corrected hiPSCs (Cre26, Cre33) is comparable to that in cord blood mononuclear cells (CBMNCs). However, the level of HBB expression in βS/βA SCD-corrected erythroblasts is >100 fold higher when compared to the uncorrected βS/βS SCD hiPSC-derived erythroblasts, even though this is >100 fold lower than that in CBMNCs.

In summary, we have successfully demonstrated site-specific correction of sickle cell disease (SCD) mutations at the endogenous HBB locus of patient-specific hiPSCs, using HBB-specific TALENs. SCD-corrected hiPSC lines showed gene conversion of the mutated βS to the wild-type βA in one of the HBB alleles, while the other allele remained a mutant phenotype. After Cre recombinase-mediated excision of the loxP-flanked DNA cassette from the SCD-corrected hiPSC lines, we obtained secondary heterozygous βS/βA hiPSCs, which express the wild-type (βA) transcript to 30-40% level as compared to uncorrected (βS/βS) SCD hiPSCs when differentiated into erythroid cells. Furthermore, we have also shown that TALEN-mediated genetic correction of disease-specific hiPSCs did not induce any off-target mutations at closely related sites. The next step is to translate these findings into pre-clinical research.

Antony Rosen, M.D.
Johns Hopkins University
2012 Exploratory Award Budget: $230,000
Disease Target: Multiple

Using hESCs to Define Novel Scleroderma Autoantigens in Stem Cells and Vascular Progenitors

Our question focused on whether stem cells and/or their differentiated progeny expressed scleroderma autoantigens at high levels, and might be the target of the immune response in this autoimmune disease. Since damage in scleroderma is focused on/around blood vessels, we used Western blotting to screen 100 scleroderma patient sera and 33 controls against the embryoid body vascular progenitors (EBVPs) and HeLa cell lysate. Serum dilutions of 1: 5,000 were used to visualize high titer antibodies. Three recurrent bands of interest at 33kD, 55kD, and >>200kD were detected uniquely in EBVPs but not in HeLas. The signals from these novel bands by immunoblotting suggested that identification using 2-D gels and MS sequencing would pose a significant challenge. We therefore sought to define whether novel autoantigens are expressed more robustly at other stages in ES differentiation.

In the first set of biochemical screens, we used 20 well-defined scleroderma patient sera to screen embryonic stem cells (ES) and EBs (H9) at days 3, 7, and 10 of differentiation. We initially validated the differentiation system using various markers of pluripotency and differentiation (Oct4, Nanog, tubulin 3, GFAP, N-Cadherin, CD31, and ALDH1), and appropriate loading controls. We examined the expression of multiple SSc autoantigens, associated with different patterns of clinical disease (limited vs diffuse scleroderma). We generated multiple preps of EBs so that we could define the variability between different experiments. The 2 preps behaved very similarly. Interestingly, topoisomerase-1 levels (antibodies to topoisomerase-1 associated with interstitial lung disease and variable severity of skin disease) increased at Days 7 and 10 in differentiating EB's, relative to ES and day 3 EBs. In contrast, NPM1 levels were higher in ES cells than in any day of EB differentiation.

We next focused on optimizing the methods for embedding human EB's for immunohistochemical analysis. Our goals were to retain as many EBs as possible during the embedding process, maintain their structure, and disperse them throughout the pellet so that distinct features of the EB's could be viewed with clarity. We selected and optimized markers of the various EB germ-layers as above. Interestingly, topoisomerase-1 appears to be most highly expressed in cells with markers of ectoderm in day 7 EBs. Studies to examine the preferential expression sites of other scleroderma autoantigens, timing of expression, as well as some control autoantigens from other autoimmune rheumatic diseases, are currently underway.

We have also recently identified expression of a prominent scleroderma autoantigen in EBVPs. Interestingly, immune responses to this antigen are associated with vascular disease and events in scleroderma patients. A manuscript describing these findings is in preparation.

Mingyao Ying, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2012 Exploratory Award Budget: $230,000
Disease Target: Parkinson's Disease

Highly Efficient Conversion of Human Stem Cells to Dopaminergic Neurons by Proneural Transcription Factor Atoh1

Since July 2012, this funding has led to one publication, one patent application and two oral presentations in 2012 & 2013 Maryland Stem Cell Research Symposium. Major discoveries from this project are summarized as below:

In **Aim 1**, we proposed to study the functions of proneural transcription factor Atoh1 in the neuronal differentiation of human pluripotent stem cells (PSCs). We found that Atoh1 is induced during the neuronal conversion of PSCs and ectopic Atoh1 expression is sufficient to drive PSCs into neurons with high efficiency. Atoh1 induction, in combination with cell extrinsic factors, differentiates PSCs into functional dopaminergic (DA) neurons with >80% purity. Atoh1-induced DA neurons recapitulate key biochemical and electrophysiological features of midbrain DA neurons, the degeneration of which is responsible for clinical symptoms in Parkinson's disease (PD). Atoh1-induced DA neurons are sensitive to neurotoxin 6-OHDA treatment, thus providing a reliable disease model for neurotoxin-induced neurodegeneration in PD. Overall, our results determine the role of Atoh1 in regulating neuronal differentiation and neuron subtype specification of human PSCs. Our Atoh1-mediated differentiation approach will enable large-scale applications of PD-patient-derived midbrain DA neurons in mechanistic studies and drug screening for PD. A manuscript describing these findings has been published on Stem Cells Translational Medicine. A patent application has also been filed.

In **Aim 2**, we proposed to determine the in vivo function of Atoh1-induced DA neurons in PD animal models. We have successfully established a PD mouse model, and we have transplanted Atoh1-induced DA neurons from human induced PSCs into these PD mice. By 8 weeks after transplantation, Atoh1-induced DA neurons survived in mouse brain and expressed DA neuron markers. We are currently testing the therapeutic effects of Atoh1-induced DA neurons in a large cohort of PD mice.

In addition, we have successfully used next-generation sequencing methods to identify Atoh1-regulated gene targets in human PSCs. We identified 925 and 375 genes that are directly induced or inhibited by Atoh1, respectively.

Further analysis found for the first time that Atoh1 can activate genes essential for DA neuron maturation. These results have led to a novel hypothesis that Atoh1 may promote DA neuron maturation, which is currently studied in our laboratory. With the generous support from MSCRF, we have established a highly efficient method to generate DA neurons from human stem cells. This method provides an innovative technique to produce highly pure human neurons, and harbors significant translational potential in disease modeling and cell replacement therapy for PD and other neurological disorders. We are currently collaborating with GlobalStem, Inc, a Maryland biotech company, to translate this innovative technique into new products that will advance the field of regenerative medicine and benefit biotechnology in Maryland.

Ola Awad, Ph.D.
University of Maryland, Baltimore
Mentor: Ricardo Feldman, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Gaucher's Disease (GD) / Neurodegenerative Diseases

Role of Autophagy Dysregulation in the Development of Neurodegeneration Using iPSC Model of Gaucher's Disease

Gaucher's disease (GD) is the most common lysosomal storage disease (LSD). GD is caused by mutations in GBA1 gene, which encodes the lysosomal enzyme glucocerebrosidase (GCase). Neuropathic form of GD is associated with neurodegeneration of the central nervous system with either rapid (type-2), or slow progression (type-3). Because recombinant GCase enzyme cannot cross the blood brain barrier, there is currently no effective treatment for neuropathic GD. It is also known that GBA mutation is the most common risk factor for Parkinson's disease suggesting a mechanistic link between the two disorders. The mechanism by which GBA mutation leads to neurodegeneration is currently unknown which prevents the development of new therapies. This MSCRF postdoctoral fellowship award funded this study, in which I used induced pluripotent stem cell (iPSC)-derived GD neurons to investigate mechanisms responsible for neuronal loss, as a model for neurodegenerative disorders. Our laboratory has previously generated and fully characterized Gaucher's disease-specific induced pluripotent stem cells (iPSCs) derived from all the three clinical sub-types including the neuropathic forms type 2 and 3. I have successfully differentiated control and mutant Gaucher's iPSCs lines into mature functional neurons as detected by the expression of neuronal specific markers and electrophysiological properties. Using this model I was able to discover a novel mechanism that underlines lysosomal dysfunction in GD.

This mechanism involved altered expression of transcription factor EB (TFEB), the master regulator of lysosomal and autophagy genes. My data demonstrated that neuropathic but not non-neuropathic GD neurons exhibited widespread lysosomal depletion and decreased expression of lysosomal markers. We also detected defective autophagic clearance marked by accumulation of autophagosomes and ubiquitin-binding protein, p62/SQSTM1 in neuropathic GD neurons, in both basal and autophagy inducing conditions.

Treatment of GD neurons with recombinant GCase (rGCase) enzyme reverted the lysosomal depletion and autophagy block indicating that GCase activity is required for maintaining a functioning pool of the lysosomes. To determine whether the reduction in lysosomal numbers in GD iPSC-NCs is caused by decreased lysosomal biogenesis, we examined the expression of TFEB. Both TFEB mRNA and protein levels were significantly decreased in neuropathic GD neurons.

Moreover, TFEB lysosomal target genes were also significantly down regulated in GD neurons. Surprisingly, lentiviral introduction of TFEB into neuropathic GD neurons failed to restore the lysosomal numbers. However, treatment with rGCase resulted in increased TFEB nuclear ranslocation and upregulated TFEB target genes in neuropathic GD neurons. This is the first report on the involvement of GCase enzyme in regulating lysosomal biogenesis potentially by acting upstream of TFEB.

Our study provides new insights into the mechanisms of lysosomal dyfunction and neurodegeneration in GD, which can have significant therapeutic implications. Our GD-iPSC model not only recapitulated neuropathic GD phenotype but also uncovered a new mechanism involved in the disease process. It can be used to further study disease mechanisms and as a platform for drug discovery.

Moreover, because many neurodegenerative diseases share similar underlying mechanisms, our study will shed the light on other neurodegenerative diseases and the benefits of drug screening can extend beyond Gaucher's disease. A manuscript describing this study is in preparation for submission to the prestigious scientific journal, PNAS (Proceedings of the National Academy of Science).

Su Mi Choi, Ph.D.
Johns Hopkins University
Mentor: Yoon-Young Jang, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Liiver Cirrhosis

Patient Specific Stem Cell based In Vitro Model of Liver Cirrhosis

Liver fibrosis will eventually lead to liver cirrhosis which is one of the top ten causes of death in the world. Currently there is no effective drug treatment for liver cirrhosis patients. Animal models cannot accurately represent human pathophysiology and there have been no human cell lines established from liver cirrhosis patients to study and develop effective therapy.

Therefore we have investigated the potential for modeling liver fibrosis/cirrhosis in vitro using patient-specific induced pluripotent stem cells (iPSCs), which could provide a molecular understanding of the disease process as well as enable the discovery of novel therapies for many liver cirrhosis patients. Based upon the aims of our original proposal, we have utilized both environmental and genetic factors on multiple patient-derived iPSC lines that we have previously established to induce or increase hepatic fibrosis. Interestingly, we have observed noticeable fibrotic changes from hepatic cells derived from patient-iPSCs compared to controls (i.e. untreated iPSCs or healthy donor-derived iPSCs) even in the absence of such factors. This could serve as a novel cellular model of human liver fibrosis.

We further evaluated the expression of multiple liver cirrhosis-associated factors in our patient-derived iPSC lines and their hepatic derivatives. We were able to determine one or more signaling pathways are critical for disease phenotype (i.e. fibrosis). To establish a sensitive reporter system enabling early and quantitative detection of hepatic fibrosis, we have generated reporter lines which can be used for monitoring Type 1 collagen expression changes. In addition, we have demonstrated the feasibility of patient iPSC-based drug screening for novel treatment of a chronic liver disease (i.e. alpha-1 antitrypsin deficiency, which can ultimately become liver cirrhosis). Through a large-scale drug screening, five clinical drugs were identified to reduce the disease feature in patient iPSC-derived hepatocyte-like cells (Choi et al., Hepatology 2013). In summary, during the last two years of my research supported by MSCRF fellowship, we have developed iPSC based in vitro models of multiple chronic liver diseases including liver fibrosis. These models will be used for studying disease pathogenesis and drug discovery for liver cirrhosis patients without therapeutic options.

Eunchai Kang, Ph.D.
Johns Hopkins University
Mentor: Guo-Li Ming, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Schizophrenia

Modeling of Major Mental Disorders Using Human Induced Pluripotent Cells Derived from Patients with a Defined Disc1 Mutation

Schizophrenia (SCZ) is a devastating mental disorder with a prominent genetic basis. Many candidate genes associated with increased risk for SCZ have been identified that appear to support a neurodevelopmental origin for this disorder. Following the initial discovery of DISC1 as a susceptibility gene for major mental disorders, including SCZ, intensive studies in rodents have implicated DISC1 in different aspects of neurodevelopmental processes in both the embryonic and adult brain. However, it remains to be elucidated how DISC1 is mechanistically linked to disease. Applying knowledge from rodent model systems to pathological human development can be very challenging. iPSCs reprogrammed from somatic cells of healthy subjects, and patients with defined DISC1 mutations offer an extraordinary opportunity to understand the function of DISC1 in human neurodevelopment and reveal novel etiological markers of SCZ.

We have generated induced pluripotent stem cells (iPSCs) derived from skin fibroblasts of SCZ and schizoaffective disorder (SAD) patients with a 4 bp deletion in DISC1, and also from healthy individuals without DISC1 mutation from the same American family. This 4bp deletion in DISC1 causes a frame-shift mutation, leading to a transcript encoding a truncated protein with nine abnormal c-terminal amino acids. The contribution of this mutation to risk for psychiatric disease and aberrant neuronal development in humans is unknown, and was the focus of this research project.

In our study, we differentiated iPSCs from patients with the DISC1 mutation and healthy family members without the mutation into human neural progenitor cells (hNPCs). Using these patient-derived hNPCs, we performed pilot transplantation studies into the hippocampus of adult mice, an endogenously proliferative region in the mature brain. Our preliminary data suggest that differentiated neurons derived from patients with the DISC1 mutation exhibit aberrant morphological development. Similar to another study using iPSCs from sporadic schizophrenia patients, we also found that the DISC1 mutant lines showed abnormalities in synaptic marker expression and electrophysiological properties. To determine the causal role of the 4bp deletion in specific phenotypes, we developed targeted genetic rescue lines and novel mutation lines using the transcription activator-like effector nuclease (TALEN) method. Restoration of the 4bp in mutant lines rescued the abnormal synaptic marker expression and deficits in electrophysiological properties, whereas introduction of the mutation in control lines led to similar synaptic abnormalities as we observed in patient lines with and endogenous DISC1 mutation.

We are currently optimizing conditions for transplantation of hNPCs into dentate gyrus of adult mouse hippocampus. These studies were critical to begin to identify how a specific human mutation associated with risk for psychiatric disease can affect the function and development of human neurons. This approach serves as a model for using human iPSCs to identify cellular phenotypes that can be used in diagnostic screening and as a discovery tool to identify novel pathways and targets for the development of new therapeutics.

Chang-Mei Liu, Ph.D.
Johns Hopkins University
Mentor: Fengquan Zhou
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Neurological Diseases

Small RNA Regulation of GSK3 Expression Modulates Human Neural Stem Cells Proliferation and Differentiation

Glycogen synthase kinase 3 (GSK3) has been shown to be associated with many of these neurological diseases. Inhibition of GSK3 with pharmacological inhibitors is emerging to be a key approach in controlling human embryonic stem cell neural differentiation or generating induced pluripotent stem (iPS) cells. Moreover, several recent studies show that GSK3 signaling is a master regulator f mouse NSC homeostasis. These studies suggest that modulation of GSK3 activity is an important mechanism for regulation of stem cell properties. To date, the role of GSK3 signaling in regulation of hNSC remains unexplored. MicroRNAs (miRNAs) are non-coding RNAs that function as epigenetic regulators of a variety of biological processes, including proliferation, differentiation and maintenance of stem cells. Many mi-RNAs, such as let-7, miR-124, miR-9, miR-25, miR-134 ,miR-137, and miR-184, have been shown playing important roles in regulation of NSCs. However, the underlying molecular mechanisms by which these miRNAs control NSCs, especially hNSCs, are not fully understood. We hypothesize that regulation of GSK3 by microRNAs plays an important role in controlling hNSC self-renewal and neuronal differentiation. Thus, in this proposed study we will 1) identify and verify miRNAs that can modulate GSK3 expression in hNSCs, and 2) determine if and how mi-RNAs regulate self-renewal and neuronal differentiation of hNSCs via modulation of GSK3 expression. In order to finish the aims, we firstly developed mouse embryonic neural stem cells. Using luciferase reporter system and electroporation technology, we found GSK3 expression is modulated by mi-RNAs pathway in mouse embryonic NSCs. And also several potential microRNAs were confirmed to target GSK3 in embryonic neural stem cells. The following are the progress reports one by one.

1. Construct of human 3'-UTR of GSK3α/β

Our preliminary data shows that GSK3 was modulated by miRNA pathways. In order to further identify miRNAs targeted to GSK3, we constructed mouse and human 3'-UTR of GSK3α/β and then sequenced. We constructed the right constructs 3'-UTR of GSK3α/β.

Construction of mouse GSK3a 3'UTR:

Mouse cDNA was used to clone 584bp mouse 3'UTR. Primers were linked with XhoI (forward: tcgCTCGAGGGGCCCCACCGACTACCCCTCCAC) and NotI (reverse: CCAGCGGCCGCGCCAATAATTTATTGAACAGAGGTCTG). The fragment was inserted into the vector psiCHECK2, we obtained the mouse GSK3a-3'-UTR construct. We used same vector psiCHECK2 for human GSK3β 3'-UTR. 4.8kb primers were designed and for human GSK3α/β 3'UTR: h.GSK3b.F4849tcgCTCGAGACAGTCCCGAGCAGCCAGCTGh.GSK3b.R4849 CCAGCGGCCGCTTTTAATTTCATTTCCAGGCTAACCTAC Human genomic DNA was used as PCR template.

2. GSK3 expression is modulated by miRNAs in mouse NSCs

We then used the software TargetScan 6.1 to predict the potential mi-RNAs that have binding sites for the 3'-UTR of GSK3α/β . To test if GSK3 is the direct target of these predicted miRNAs, we cloned 3′ untranslated regions (UTRs) of GSK3 directly from mNSCs cDNA and generated a dual luciferase 3′ UTR reporter construct. Using this reporter, in addition to miR-92b, miR-221, miR-222, we also found that overexpression of RNA oligo mimics miR-26a, miR-135a, miR-135b and miR-101a in mouse NSCs could suppress the GSK3β 3′ UTR luciferase reporter activity, indicating that these miRNAs may bind to the GSK3β 3′ UTR and regulate GSK3 expression. We will further test the results by western blotting after electroporation RNA oligo mimics into neural stem cells. We are also working on finding more potential miRNAs that target GSK3, with special focus on miR-28 and miR-708 which are predicted to target both Gsk3a and Gsk3b-3'-UTR.

3. miR-92b and miR-221, miR-222 promote NSC proliferation

To identify the functions of the selected microRNAs, we over expressed three miRNAs mimics miR-92b, miR-221 and miR-222 in neural stem cells. By using BrdU pulse experiments, we found that over expression of these three miRNAs significantly increased the NSC proliferation. Because inhibition of GSK3 promotes NSC proliferation, our results suggest that miR-92b, miR-221, miR-222 regulate NSC proliferation via modulation of GSK3 expression. For the other selected miRNAs, we are testing the functions in neural stem cells.

4. Construction of vector-based miRNA expression plasmids

To explore the functions of mircroRNAs that target GSK3 3'UTR in vitro and in vivo, we designed miRNA cloning primers linked with single cut restriction enzymes for cloning miRNA expression sequences into the CMV-GFP plasmid vector. We already generated the expression microRNA vectors, miR-28a, miR-135a, miR-135b, miR-23b, miR-29a, miR-29b. We will test these plasmid expressed micoRNAs' functions in neural stem cells in proliferation and differentiation in vitro. Then test in vivo in E14.5 embryos by electroporation to confirm the functions in neural stem cells.The cloned miRNA will be confirmed by sequencing. We are still working on cloning more miRNAs expression vectors.

5. Development of lentivirus-based constructs for gain- and loss of function studies of miRNAs

To over-express mi-RNAs in NSCs and human neural stem cells, we constructed a mi-RNA expression vector in a lentiviral vector backbone with a GFP marker for example, miR-92b. We also constructed a miR-sponge vector to down regulate the endogenous mi-RNAs. Based on the results, we are working on more miRNAs lentivirus expression vectors, miR-26a, miR-135a, miR-135b, miR-101a. By using packaged lentivirus expression system, we will enhance the transfection efficiency in human neural stem cells and will perform further studies to answer whether the miRNA functions via GSK3 in human neural stem cells.

Georgia Makri, Ph.D.
Johns Hopkins University
Mentor: Hong Song, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Rett Syndrome

Patient-Specific iPSCs for Modeling and Treatment of Rett Syndrome

Rett Syndrome (RTT is a severe autism spectrum disorder causing progressive loss of motor and language skills that primarily affects girls between 6 and 18 months of age. Mutations in the X-linked gene MeCP2 are the primary cause of the disease. There is an array of RTT-causing MeCP2 mutations, from missense, nonsense, insertions, deletions, and splice site variations, which are dispersed throughout the gene. There is a wide range in the severity of symptoms that is influenced by the mosaic pattern of X chromosome inactivation at the cellular level. Although the primary genetic locus of this disease has been identified, the mechanisms through which this mutation exerts its effects on neurodevelopment and brain function are not well understood. For this project, I have successfully generated iPSC lines from patients with specific MeCP2 mutations and a clinical diagnosis of RTT. Using an integration-free method of reprogramming based on Sendai Virus delivery of transcription factors, we have generated iPSC lines from patients with RTT. We have fully characterized these iPSC colonies for correct karyotyping and pluripotent potential in vitro by confirming the expression of pluripotency markers including Oct3/4, SOX2, TRA-1-60, TRA-1-81, and Nanog. We also confirmed pluripotency by testing the ability of iPSCs to differentiate in vitro and in vivo (teratoma assays) into all three germ layers (endoderm, ectoderm and mesoderm). The colonies that appeared positive for pluriopotency markers, with normal karyotyping and the ability to differentiate in vitro and in vivo to all germ layers, were selected and used for all subsequent experiments in the present study.

As has been suggested in earlier studies, different mutations in the MeCP2 gene may induce different phenotypic characteristics and symptomatology. Further, the same mutation may have a different functional impact on different subtypes of neurons. Therefore, we have focused on optimizing protocols for high efficiency neuronal differentiation. We have been able to generate highly enriched populations of cortical glutamatergic neurons and GABAergic neurons. We have been able to achieve substantial numbers of dopaminergic neurons, but at a slightly lower efficiency. There were no differences in the efficacy or distribution of neuronal subtypes derived from iPSCs with or without MeCP2 mutations. I am currently following up with functional studies of these populations. Generating relevant cell types for the study of disease is critical to understand the mechanisms underlying neural dysregulation and to develop new therapeutic strategies. Because MeCP2 can perform as both a transcriptional enhancer, as well as a repressor, generation of nearly pure neuronal subtype populations will facilitate a better understanding of the function of this gene in specific human cell types.

Yi-Lan Weng, Ph.D.
Johns Hopkins University
Mentor: Guo-Li Ming, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Multiple

Effects of DNA Methylation Reprogramming in Axonal Plasticity and Regeneration

Successful axon regeneration hinges on the growth competence of injured neurons that enables severed axons to re-grow and correctly recognize their synaptic targets. Cumulative evidence indicated that a conserved group of transcriptional regulators and regeneration-associated genes (RAGs) determine the growth state of neurons and their reactivation is essential for successful axon regeneration. However, the mechanisms responsible for transcriptional activation of RAGs and initiation of regenerative processes are largely unknown.

Using iPSC-derived motor neurons, I assessed the role of DNA methylation in regulating intrinsic growth state of human neurons. I have successfully optimized the protocol for motor neuron differentiation and tested different culture time points for modeling neuronal maturation to gain insight into age-dependent axon regeneration mechanisms. We will use methyl-sensitive cut counting (MSCC) to create a genome-scale methylation profile and quantitatively compare the methylation landscapes before and after neuronal maturation (juvenile vs. adult) and with or without axotomy. I expect these results will provide correlative evidence that DNA methylation plays a critical role in regulating RAGs during neuronal maturation and after injury.

In parallel, I also use mouse adult dorsal root ganglion neurons to study epigenetic mechanisms of axon regeneration. We used qPCR and RNA in situ hybridization to establish gene expression profiles of several epigenetic regulators in intact and injured neurons. I found that members of the Gadd45 family, which have the ability to promote active DNA demethylation, were highly expressed in neurons in response to axotomy. This result is consistent with other studies under different injury conditions. I also used both HpaII/MspI digestion-based PCR assay and bisulfite sequencing to determine methylation states of RAGs. I found certain loci of RAGs can undergo DNA demethylation after injury. Importantly, inhibition of DNA demethylation pathways can impair activation of these RAGs and decreases regenerative capacity of neurons. Our data suggest active DNA demethylation is another regulatory layer in initiating axon regeneration.

Yaxue Zeng, Ph.D.
Johns Hopkins University
Mentor: Hong Song, Ph.D.
2012 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Multiple

Characterizing the Role of Active DNA Demethylation in Reprogramming of Human Somatic Cell into Stem Cells

Recent advances in stem cell research have led to the groundbreaking discovery of the ability to reprogram somatic cells to pluripotent stem cells, or induced pluripotent stem cells (iPSCs), by introducing a group of transcription factors. Although it is known that extensive cellular changes occur during the reprogramming process, the molecular mechanisms underlying these changes are largely unclear. Given the different DNA methylation profiles between pluripotent and differentiated cells, identifying the dynamic changes of DNA methylation and associated histone modifications becomes an essential key to unraveling the molecular mechanism of reprogramming. The recent discovery of the active DNA demethylation pathway, TET-APOBEC-TDG, has provided important clues to characterize key epigenetic events in multiple cellular settings. In this project, I am trying to determine the role of TET-APOBEC-TDG active DNA demethylation in the human somatic cell-iPSC conversion and identify their transcriptional targets and associated histone modifications during reprogramming. This is the second year of my fellowship award, and I have made progress in all three aims.

Aim 1. Characterize the role of TET-APOBEC-TDG pathway in somatic-iPSC or somatic-iN conversion. Progress and Discoveries: We have successfully knocked down Tet1 and TDG in human fibroblast, and are in the process of characterizing the reprogramming efficacy of those TET1-KD or TDG-KD fibroblast. We have also characterized the expression level of TET1-3, APOBEC, and TDG in different stages of reprogramming.
Obstacles: Consistency of reprogramming in each experiment is tricky, but are essential for parallel comparison of reprogramming in wild type and KD cells. I will carefully detemine the optimal dose of knocking down construct to achieve an optimal knocking down effect as well as healty cell status, therefore to make a fair comparison with control cells during reprogramming.

Aim 2. Identify pluripotency/neuronal gene targets of TET in reprogramming. Progress and Discoveries: We have collected cells from different stages of reprogramming and identified a few key genes that undergo significant changes of expression pattern. We are testing the occupancy of TET on these genes by chromatin IP, and characterizing changes of methylation status on their promoters by methylation specific enzymes. Obstacles: 1. Endogenous pull-down of TET is not ideal because of lacking a good TET1/2 antibody. We are trying to make a FLAG knock-in line of TET1/2/3 by CRISPR method so that high quality of chromatin IP can be achieved. 2. Screening of CpG sites undergoing methylation change is labor and time-intensive. Therefore we are using methylation specific cutting instead of previously proposed bisulfate sequencing to identify target loci. Also analysis of previous whole-genome bisulfate sequencing database will be helpful to narrow down candidate loci.

Aim 3. Evaluate the crosstalk between DNA modifications and histone modifications on pluripotency genes during the reprogramming process.Progress and Discoveries: We have characterized expression levels of different histone modifiers during reprogramming and are quantifying active and repressive histone markers on several pluripotent genetic loci during reprogramming. We have also generated effective shRNA constructs against TETs, EZH2 (H3K27 methyltransferase) and G9a (H3K9 methyltransferase). By comparing gene expression profile of fibroblast or iPSC with deficiency of these epigenetic modifiers, we are determine their common gene targets and will evaluate their roles on changes of both histone modifications and DNA methylation on those targets during reprogramming. Obstacles: candidate approach to identify common gene targets of histone /DNA modification during reprogramming is not very efficient. To identify target genes in a non-biased way, we are collaborating with bioinformatics experts and trying to get a complete list of candidate genes.

Translational Potential: Our study delineates a systematic investigation of epigenetic machinery in reprogramming and is essential for the development of a complete and accurate picture of the functional capacity of derived stem cells. Furthermore, our study will provide a mechanistic basis for further improvement of iPSC/iN reprogramming strategies, therefore can potentially lead to advances in regenerative medicine and modeling human disease by patient-derived iPSC/iN.

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