MSCRF

Maryland

STEM CELL RESEARCH FUND

ACCELERATING CURE

Annual Report

Fiscal Year
2016

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

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Baltimore County

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Georgetown University Medical Center.

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Review Board, Johns Hopkins Bloomberg School of
Public Health; Embryonic Stem Cell Research Oversight
Committee, Johns Hopkins School of Medicine.

Marye D. Kellermann, RN, Ph.D.
(Appointed by the Speaker of the House of Delegates)
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Enterprises NECESSARY NP Reviews &
NECESSARY Workshops.

Sharon Krag, Ph.D.
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Bloomberg School of Public Health.

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Johns Hopkins Berman Institute of Bioethics;
Assistant Professor, Dept. of Pediatrics,
Johns Hopkins School of Medicine.

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Early & Active Supporter of Biotech Companies

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

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

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

Congratulations to the 2016 MSCRF Grant Award Recipients

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Dr. Peter Altman
BioCardia, Inc.
Heart Failure Trial

Investigator Initiated Grant Awards:

Dr. Christopher Chiang
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Johns Hopkins University
Single Cell Analysis of Hippocampal Neurogenesis Defects in Kabuki Syndrome 1

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Use of pH sensitive MRI Contrast Producing Hyaluronic Acid Scaffolds for MSC Therapy of IVD

Dr. Guo-Li Ming
Johns Hopkins University
A Diametric Model of Neurodevelopment and Pathophysiology of Schizophrenia and Autism

Dr. Gordon Tomaselli
Johns Hopkins University
Tissue Models of Heritable Cardiac Arrhythmias

Exploratory Grant Awards:

Dr. Cynthia Berlinicke
Johns Hopkins University
Single Cell Transcriptomic Analysis of Stem Cell-Derived Retinal Cups

Dr. Peter Calabresi
Johns Hopkins University
Function and Myelination of Human Retinal Ganglion Cells Bearing Polymorphisms Derived from the MS-A

Dr. Amy DeZern
Johns Hopkins University
Regenerative Medicine to Restore Normal Hematopoiesis in Aplastic Anemia

Dr. Tami Kingsbury
University of Maryland, Baltimore
Eyes Absent-1 (EYA1) as a Novel Hematopoietic Stem-Progenitor Cell Regulator

Dr. Vasilki Machairaki
Johns Hopkins University
Human iPSC-Derived Extracellular Vesicles: Targets for Novel Diagnostic and Therapeutic Strategies

Dr. Cyrus Mintz
Johns Hopkins University
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Dr. Enid Neptune
Johns Hopkins University
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Dr. Giorgio Raimondi
Johns Hopkins University
Engineering a “Hybrid Thymus” to Promote Transplant Tolerance and Graft-Protective Immunity

Dr. Chinmoy Sarkar
University of Maryland, Baltimore
Neuronal Differentiation of iPS Cells by Autophagy Induction in Oxidative Environment to Treat TBI

Dr. Kathryn Wagner
Hugo W. Moser Research Institute at Kennedy Krieger
A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation (Continuation)

Post-Doctoral Fellowship Grant Awards:

Dr. Allison Bond
Johns Hopkins University
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Evaluating the Impact of Genetic Risk Factors for Psychiatric Disorders on Interneurons

Dr. Rebecca Fawcett
University of Maryland, Baltimore
Mentor: Dr. Steven Bernstein
Analysis of the Newly Identified Human ONL Stem Cells and their role in Glaucoma

Dr. Miguel Flores-Bellver
Johns Hopkins University
Mentor: Dr. Maria Valeria Canto-Soler
3D Neural Retinal/RPE Complex from Human iPS Cells: A Novel Age-related Macular Degeneration System

Dr. Ziyuan Guo
Johns Hopkins University
Mentor: Dr. Hongjun Song
Role of Structural and Functional Changes of Dendritic Spines in Patient-Derived C9ORF72 iPS Neurons

Dr. Hyunhee Kim
University of Maryland, Baltimore
Mentor: Dr. Valina L. Dawson
Parthanatos in Parkinson’s Disease

Dr. Srinivasa Rao Sripathi
Johns Hopkins University
Mentor: Dr. Donald J. Zack
Modulation of RPE Epithelial Mesenchymal Transition as an Approach for Treatment of AMD

Dr. Dhruv Vig
Johns Hopkins University
Mentor: Dr. Sean X. Sun
Geometric Cues in the Establishment and Maintenance of Heterogeneous Stem Cell Colonies

Dr. Qingfeng Wu
Johns Hopkins University
Mentors: Dr. Sean Sun & Dr. Sharon Gerecht
Using microorganoids to Model the Effects of Maternal Stress on the Hypothalamic-Pituitary System

Maryland Stem Cell Research Fund Grants - Completed

Closed: 2012 MSCRF Award:

Dr. Amnon Bar-Shir
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Jeff Bulte
Mri-Based Reporter Genes for Non-Invasive Assessment of the Fate of Stem Cell-Seeded Scaffolds

Dr. Jeff Bulte
Johns Hopkins University
Investigator Initiated Award
Co-Encapsulation of Human Mesenchymal Stem Cells and Islet Cells for Treatment of Type 1 Diabetes

Dr. Christopher Donnelly
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Jeffrey Rothstein
Development of an Antisense Oligonucleotide Therapeutic Utilizing Stem Cell Derived Patient Astrocytes to Treat ALS and Dementias Caused by C9ORF72 Expanded Hexanucleotide Repeat

Dr. DaWei Gong
University of Maryland, Baltimore
Investigator Initiated Award
Towards Modeling Pathogenesis and Treatment of Congenital Generalized Lipodystrophy Using Patient-Specific iPSCs

Dr. Pinar Huri
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Warren Grayson
Engineering Clinically-Applicable Vascularized Bone Grafts using Adipose-Derived Stem Cells

Dr. Hongkai Ji
Johns Hopkins University
Exploratory Award
Global Prediction of Transcription Factor Binding Sites in Lineage Specific Neuronal Differentiation

Dr. Gabsang Lee
Johns Hopkins University
Investigator Initiated Award
Derivation of Functional Nociceptive Neurons From hESC and Its Application to Pain-Disorder Human iPSC

Dr. Guo-li Ming
Johns Hopkins University
Investigator Initiated Award
Toward Correction of Neurodevelopmental Defects Of Neurons Derived from Patients with Mental Disorders

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

Dr. Steven Zhan
University of Maryland, Baltimore
Exploratory Award
Modulation of Homing and Engraftment of Hematopoietic Stem Cells by I-BAR Proteins

Closed: 2013 MSCRF Awards:

Dr. Peter Andersen
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Chulan Kwon
Identification of chamber-specific Cardiac Progenitor Populations

Dr. Jing Cai
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Duojia Pan
Hippo Signaling in Intestinal Stem Cell Homeostasis and Carcinogenesis

Dr. Hugo Guerrero-Cazares
Johns Hopkins University
Exploratory Award
Controlling Migration of Human-Derived Fetal Neural Stem Cells Via Slit Proteins in a Demyelination Animal Model

Dr. Xiaofeng Jia
University of Maryland, Baltimore
Exploratory Award
Electrical Stimulation on Neural Crest Stem Cell Transplantation in Nerve Regeneration

Dr. Sunjay Kaushal
University of Maryland, Baltimore
Investigator Initiated Award
Characterization of Resident Cardiac Stem Cells in Neonates

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

Dr. Kathryn Wagner
Hugo W. Moser Research Institute at Kennedy Krieger
Exploratory Award
A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation

Dr. Jinchong Xu
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Valina Dawson
Cell Replacement for Stroke Using hiPSC Derived 3D-Organized Cortical Neurons

Closed: 2014 MSCRF Awards

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

Dr. Stephen Eacker
Johns Hopkins University
Exploratory Award
Translational Targets of mTOR in hNPC Development

Dr. Sonia Franco
Johns Hopkins University
Exploratory Award
Induced Pluripotent Stem (iPS) Cell-Based Approaches for Modeling and Treating Ataxia-Telangiectasia

Dr. Anna Jablonska
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Piotr Walczak
Genetic Engineering of Glial Progenitor Cells for Improved Intra-Arterial Targeting In Stroke

Dr. Raju Khatri
University of Maryland, Baltimore
Post-Doctoral Fellowship Award
Mentor: Dr. Michal Zalzman
Increasing the Replicative Lifespan and Quality of Adult Mesenchymal Stem Cells

Dr. Sang Hoon Kim
Johns Hopkins University
Post-Doctoral Fellowship Award
Mentor: Dr. Hongjun Song
Modeling and Characterization of Double Cortex Syndrome using iPSCs and Cerebral Organoids

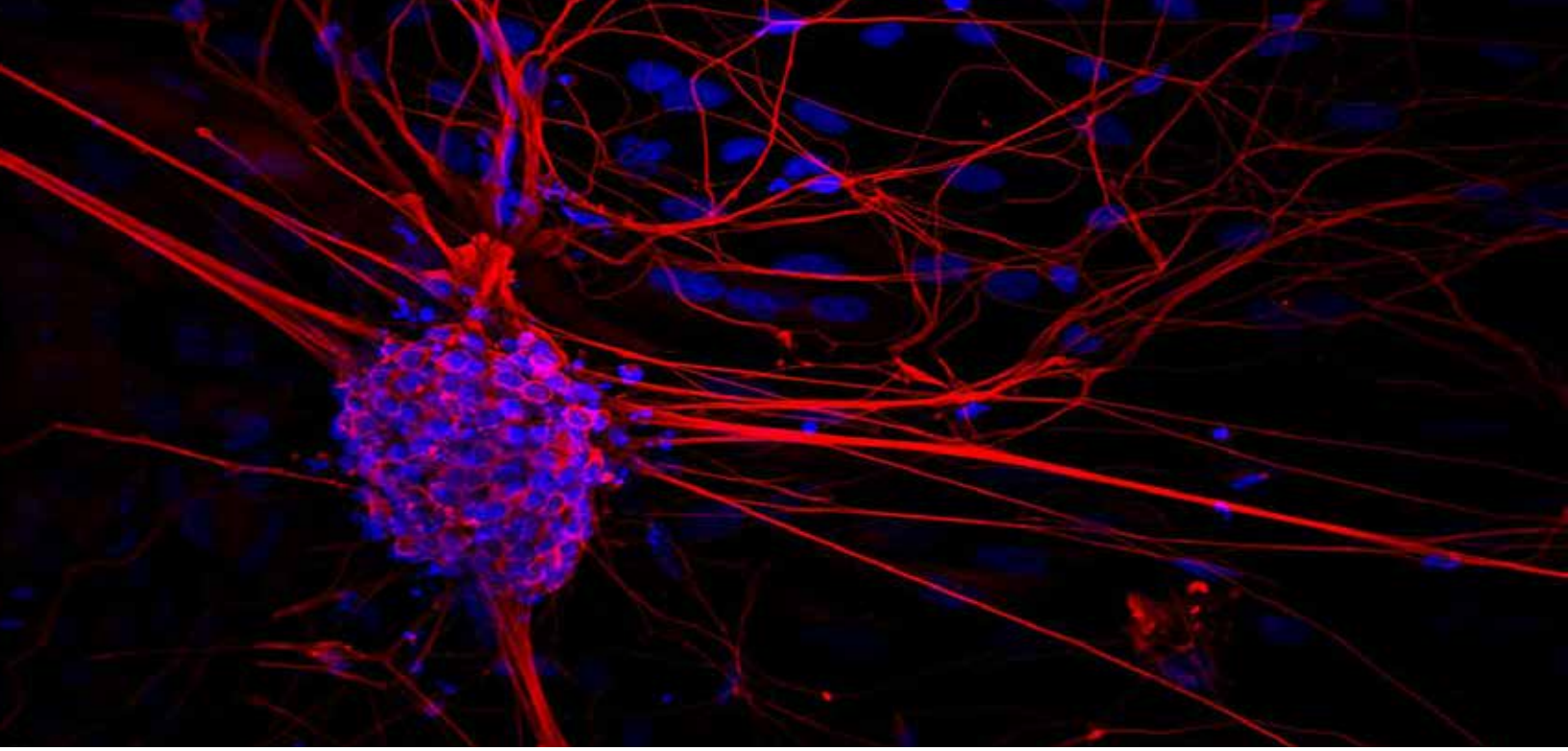
Dr. Marta Lipinski
University of Maryland, Baltimore
Exploratory Award
Modeling Parkinson's Disease Function of the PARK10 Gene USP24 in Human iPS Cells

Dr. David Nauen
Johns Hopkins University
Exploratory Award
Investigating Mechanisms of Epileptogenesis Using Human Induced Pluripotent Stem Cells

Dr. Tea Soon Park
Johns Hopkins University
Exploratory Award
Treatment of Diabetic Retinopathy with Human iPSC-Derived Vascular Progenitors

Dr. Feyruz Rassool
University of Maryland, Baltimore
Exploratory Award
Efficiently Reprogramed Cells with a MYC Signature Display High Fidelity Repair of DNA Damage

Dr. Joseph Stains
University of Maryland, Baltimore
Exploratory Award
The Role of the Beta-Catenin Signaling Cascade in the Skeletal Phenotype of Hutchinson-Gilford Progeria Syndrome



Maryland Stem Cell Research Fund

Over the last nine years the Maryland Stem Cell Research Fund has funded basic and translational research projects in universities and in companies. The advancement of the field during those years has been tremendous and our understanding of the science, the cells, and their effect on multiple diseases has improved by order of magnitude. The field of regenerative medicine represents a remarkable leading innovation in medical care. Embracing principles of stem cell technology and tissue engineering, combining advanced biomaterials and biologics, to replace or regenerate human tissues and organs and restore their functions, are just a few of the things regenerative medicine will enable.

Stem Cell Expert Advisory Board

To better understand and evaluate the potential of our awards, we invited world leaders in the field of stem cell for a full day of review and evaluation of our current and past funded awards. This group has agreed to continue to volunteer and periodically review our progress and advise our awardees of the best path to move forward.

National and International Partnering

Throughout the year the MSCRF executive director was invited to represent the state of Maryland in business and scientific stem cell meetings. In those meetings we were able to establish collaboration and partnerships with many other organizations from the Israel Stem Cell Society, the Canadian Center for Regenerative Medicine, the California Institute of Regenerative Medicine, NYSTEM in New York and more. Establishing and maintaining those relationships will allow our funded awardees to leverage funding as well as move forward with their development.

Stem Cell Symposium and Partnering

The MSCRF partnered with the Tech Council of Maryland to produce Bio+Tech16. This life sciences and technology conference brought together more than 600 professionals in both fields so that they may meet and forge new partnerships that will lead to great new discoveries. This large two day conference showed the potential of joining our own core of researchers, scientists, physicians and entrepreneurs to grow the industry.

Fostering Discovery and Cures

In 2016, MSCRF has invested in research spanning multiple tissue systems and a wide range of disease areas including schizophrenia, parkinson's disease, autism, kabuki syndrome, cardiac arrhythmias/ heart disease, muscular dystrophy, ischemic/traumatic brain injury, airway disorders, macular degeneration and glaucoma. We have brought together scientists in academic and industrial settings to innovate and combine cutting-edge technologies in biomedical research and engineering to push the boundaries of human stem cell-based disease modeling and cellular therapies. The progress our 2015 awardees have made exemplify our mission and these initiatives. Our pre-clinical grant recipient MaxCyte Inc is advancing the development of gene-editing therapies for patients with rare genetic diseases such as Chronic Granulomatous Disease. Our discovery grant recipient, Dr. Luis Garza is advancing the clinical trial of human fibroblast stem cells to convert skin identity and enhance prosthetic use, research that promises to improve quality of life of amputees.

Dr. Nicholas Maragakis is developing imaging biomarkers for stem cell transplantation in Lou Gehrig's disease (ALS). Dr. Ricardo Feldman and his team have furthered our understanding of the molecular mechanisms that lead to neurodegenerative disorders such as Gaucher and Parkinsons's disease and are identifying novel biotherapeutics for GBA1-associated neurodegeneration. Drs. Hongjun Song and John Fisher are applying advances in engineering technologies to develop methods to expand and model organ systems thereby accelerating regenerative medicine applications. Dr. Chulan Kwon and his colleagues have advanced our understanding of the mechanisms of human heart precursor renewal and maturation. The work of these and other scientists we support emphasize that the aim of our collective research is to advance human stem cell research and treat disease.

Accelerating Cures

The Maryland Stem Cell Research Commission announced, a few months ago, the launch of a new initiative called Accelerating Cure. This new initiative will support regenerative medicine and cell therapy technologies moving from research to commercialization and finally to clinical trials. Accelerating Cure will have five programs to address the different stages the technologies need to go through.

TEDCO through the Maryland Stem Cell Research Fund will manage those programs and the Maryland Stem Cell Research Commission will continue to establish criteria, standards and requirements to ensure that all projects comply with state law.

Accelerating Cure's five programs will support the stages a regenerative medicine technology has to go through to make it to market, from Discovery to Validation to Commercialization and through Clinical Trials, as well as keeping and expanding the post-doctoral Program. The five new programs are:

Discovery:

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

Validation:

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

Commercialization:

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

Clinical:

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

Post-Doctoral Fellowship:

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

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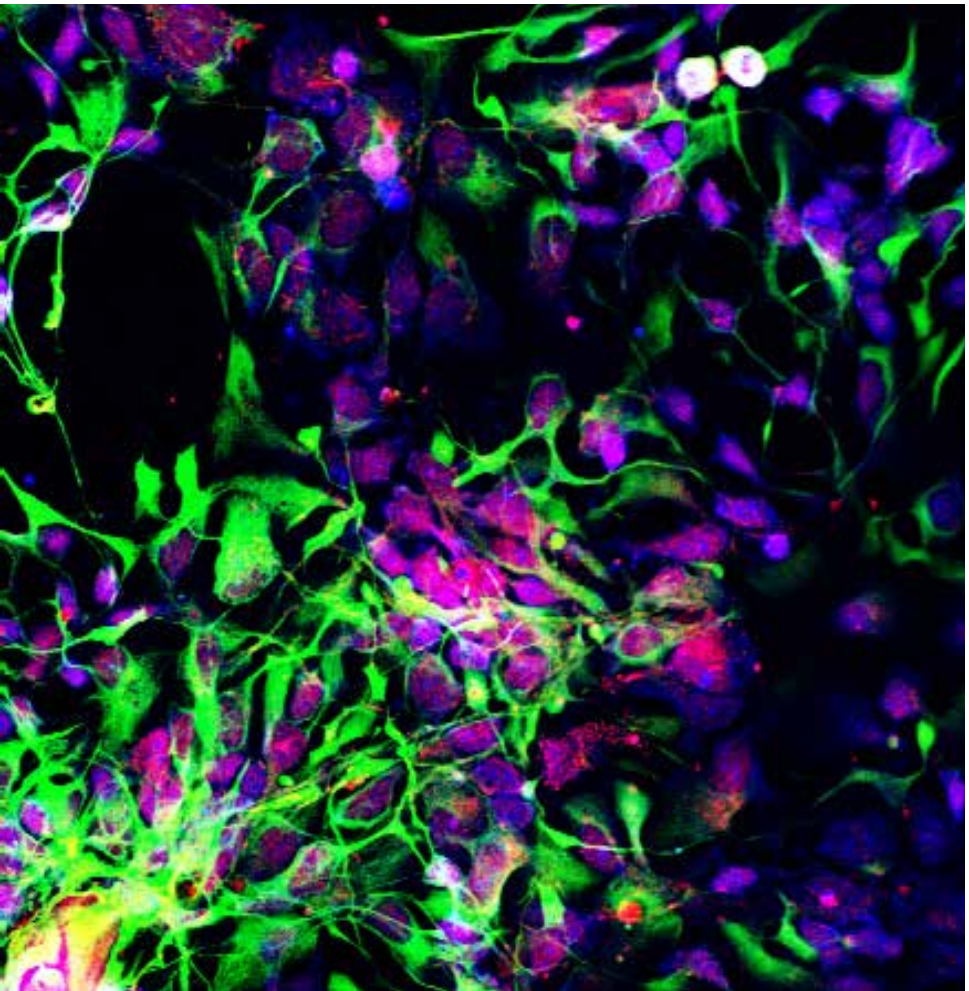
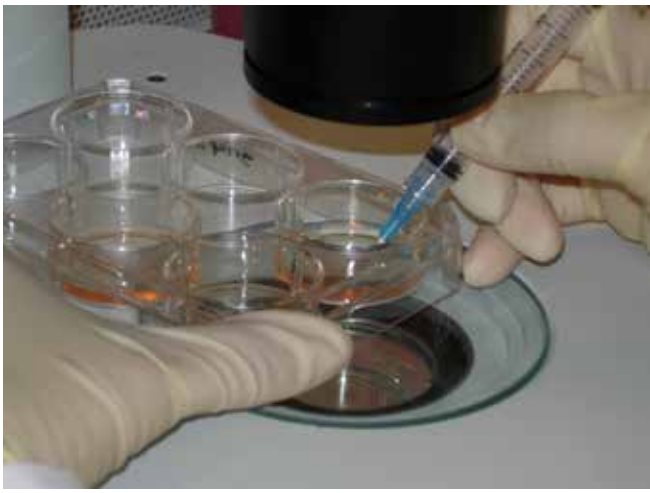
Pre-Clinical Research Grant Award:





Peter Altman, Ph.D.
BioCardia, Inc.
Award Amount: $750,000 (1:1 Match)
Disease Target: Heart Failure

CardiAMP Heart Failure Trial

Heart failure is an enormous unmet global clinical condition for which progenitor and stem cells derived from bone marrow have shown therapeutic promise. Solutions that include culture expansion of particular cell phenotypes or the selection of a specific population for re-administration without expansion are costly for autologous cell therapy, as every dosage must be treated as a separate manufacturing lot. Our group has developed a comprehensive approach that considers patient source marrow, cell processing, and cell delivery together that has enormous promise, avoids these costs, and has a shorter pathway to being available for patients. The CardiAMP Heart Failure Trial is supported by BioCardia's preclinical, and two previous clinical studies, as well as by other clinical studies with selected cells from the marrow for similar indications. The trial will test a novel comprehensive solution for autologous progenitor and stem cell therapy that includes the CardiAMP Potency Assay, CardiAMP Cell Separator point-of-care processing platform, and the Helix transendocardial delivery system under an Investigational Device Exemption (IDE). Therapeutic benefit in prior marrow HF studies has been correlated to higher effective doses of specific stem cell types. One of the primary challenges with point-of-care autologous cell therapy is the variability of the marrow aspirate compositions (and hence cell outputs) between patients.

The Potency Assay is a novel and important element of the CardiAMP cell therapy, and assesses whether the amount of select cell types from a marrow sample meets a proprietary acceptance criteria derived from data from these past trials. The CardiAMP Potency Assay is the first of its kind proposed as a quality-based inclusion criteria for a non-cultured autologous cell therapy. The CardiAMP trial will enroll up to 260 heart failure patients at 40 centers in the US. Efficacy endpoints include change in how far patient scan walk (primary endpoint) and change in quality of life at 12 months. Safety endpoints include overall survival at 12 months, freedom from Major Adverse Cardiac Events (MACE) and time to first MACE. The trial is expected to take five quarters to enroll, and nine quarters to deliver top line data. It is anticipated to be inexpensive relative to other trials in this space as Medicare and the insurers that follow Medicare will reimburse for the trial nationally. If successful in meeting its primary efficacy and important safety endpoints, this one trial could suffice to support application for FDA pre-market approval and result in new therapy being available for heart failure

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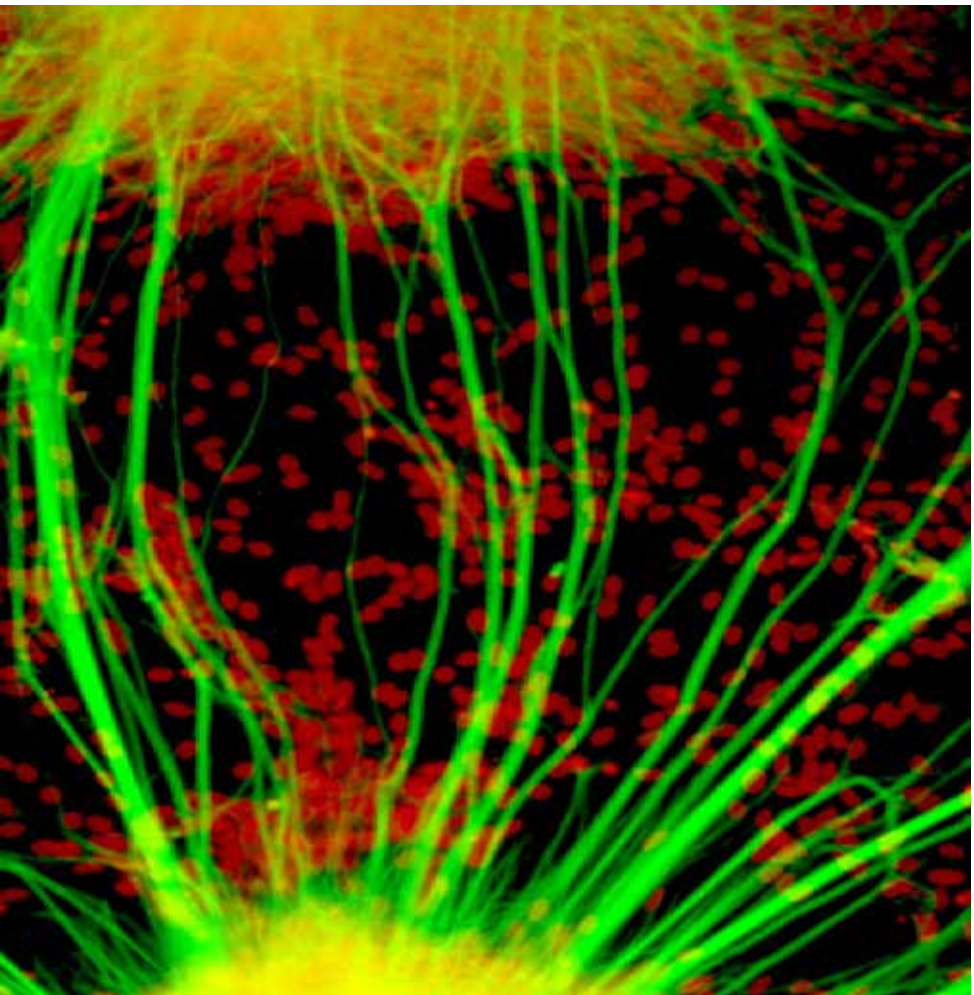
Investigator Initiated Research Grant Awards



Christopher Chiang, Ph.D.
TheraCord, LLC
Award Amount: $466,325
Disease Target: Leukemias, Lymphomas, Anemias, Cerebral Palsy, Graft-Versus-Host Disease

TheraCord Cord Blood Collection Device

Umbilical cord blood is a readily -available source of transplantable hematopoietic stem cells unhindered by ethical issues or complex surgical extraction. Cord blood (CB) is collected from the umbilical cord and placenta after birth. Cord blood transplants are used in the established treatments of over 80 diseases, similar to bone marrow transplant, but with the advantage of having increased flexibility in immunological (HLA) matching, lower incidence of graft-versus-host disease (GvHD), and immediate availability for transplant from storage. Currently there are over 670 ongoing clinical trials examining cord blood's potential use in treating diseases that collectively affect over 300 million people worldwide. Currently, cord blood is banked privately, as fee-for-service, and publicly, similar to blood donations.

Despite its potential therapeutic advantages, cord blood transplants constitute a minor fraction of hematopoietic stem cell transplants, and over 95% of cord blood in the US remains uncollected.
One of the primary reasons for the underutilization of cord blood is its ineffective collection method; the conventional, gravity-driven needle and blood bag is inefficient, highly skill-dependent, and subject to routine failure in collecting sufficient quantities of stem cells for transplantation. Because clinical outcomes are driven by cell dose per patient body weight, more than 60% of cord blood units collected in 2012 for US public banking were discarded, due to having too few cells for clinical use. Of the units banked, fewer than 10% contain enough cells to treat an adult, despite adults being the primary recipient of blood cell transplants.
To solve the inadequacies of the current collection method, TheraCord is developing a semi-automated cord blood collection device, The CBx System which utilizes placental perfusion to maximize the collection yield. The CBx System will allow staff or banks' collection specialists to reproducibly harvest high quality, aseptic collections with minimal training, time, and effort.

To date, we routinely observe a 50% increase in cell numbers obtained via the CBx System compared to routine cord blood harvesting methods. This is anticipated to increase the number of units that reach at least 17.5x108 TNC, the minimum threshold suitable for adult therapy, by 5-fold. In this project we will determine whether cord blood collection via the CBx System exhibits higher maternal cell contamination and determine the optimal post-collection strategy to maximize cell yield.

Sharon Gerecht, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $687,696
Disease Target: Diabetic Wounds

Engineered Human Vascularized Constructs to Treat Diabetic Wounds

Individuals with diabetes exhibit significant impairments in wound healing, which underlies high incidence of diabetic foot ulcers, as well as morbidity and mortality associated with this condition. Importantly, diabetes-induced abnormalities in blood vessels function likely aggravate the impairment in neovascularization in diabetic wounds. Stem cell therapy is emerging as a promising approach for wound healing in diabetes. Specifically, The use of human induced pluripotent stem cells (hiPSCs) offers the potential for a source of patient-own healthy vascular cells. We have established a novel approach to derive vascular cells from hiPSCs that can generate multicellular vascular network in a 3D hydrogel matrix. Towards autologous therapy for diabetic wound healing, we recently found similar vascular differentiation from several type 1 diabetic (T1D) hiPSC lines. When embedded in a hydrogel, vascular cells derived from T1D-hiPSC assemble into 3D networks. However, it is unclear (1) whether the T1D-vascular networks are similar to those generated from healthy hiPSCs; (2) if the T1D-vascular constructs can be delivered and integrate with the host vasculature and (3) if treating diabetic wounds with the T1D- vascular constructs would accelerate wound healing. The proposed studies address each of these unknowns. Successful completion of the proposed activities will have vast ramifications demonstrating the value of hiPSCs for vascular reconstruction and patient-specific treatment for diabetic wounds.

Michael McMahon, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Award Amount: $690,000
Disease Target: Traumatic Brain Injury

Use of pH sensitive MRI Contrast Producing Hyaluronic Acid Scaffolds for MSC Therapy of IVD

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

Guo-Li Ming, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Schizophrenia, Autism Spectrum Disorders

A Diametric Model of Neurodevelopment and Pathophysiology of Schizophrenia and Autism

Neuropsychiatric disorders, including autistic spectrum disorders (ASD) and schizophrenia (SCZ), are debilitating conditions that are postulated to have a neurodevelopmental etiology. SCZ and ASD are estimated to affect 1% of population world-wide each, costing over $60 billion and $175 billion annually in the United States alone, respectively. Because these disorders can be so debilitating, often requiring lifelong assistance, the global societal and familial burden is much greater than other diseases. Under most circumstances, the current available symptomatic treatment is only partially effective, and the response to the treatment varies from patient to patient. Therefore, development of rational therapeutics based on knowledge of the etiology and pathogenesis of the diseases is critically needed. Both schizophrenia (SCZ) and autistic spectrum disorders (ASD) have a developmental origin and are highly inheritable. Genetic mutations at a single gene or specific alterations of a relatively large section of the genome (DNA copy number variations or CNVs affecting the copy number of multiple genes in the section) have been identified as significant risk factors for ASD and SCZ. For example, 15q11.2 CNVs have emerged as prominent risk factors for various neuropsychiatric disorders, including ASD, SCZ and intellectual disability. Interestingly, 15q11.2 microduplications (3 copies of genes in this section) have been associated with ASD, whereas microdeletions (only one copy of genes in this section) of the same region have been identified as one of the three most frequent CNV risk factors for SCZ. Furthermore, a recent study show that even in normal subjects, 15q11.2 microdeletion and microduplication result in opposite effects on the volume of some brain regions that deviate from normal population. Together, these studies have established 15q11.2 CNVs as prominent dosage-sensitive genetic risk factors for neuropsychiatric disorders. Because CNVs frequently contain multiple genes, there is no good model systems to study how genetic variants like CNVs lead to aberrant neuronal development and contribute to the pathogenesis of ASD and SCZ. We have successfully generated and validated a collection of induced pluripotent stem cell (iPSC) lines from normal individuals and those with 15q11.2 microduplications or microdeletions. In our preliminary analyses of these iPSC lines, we have observed significant deficits in neural development with 15q11.2 microdeletion. In the current proposal, we propose to use patient-specific iPSCs with 15q11.2 duplication and deletion to test the hypothesis that ASD and SCZ represent diametric conditions with genetic dosage and neurodevelopmental phenotypes. We also propose to perform a high-throughput phenotypic screen to identify drugs that may reverse the cellular phenotype of neurodevelopment in cells with 15q11.2 CNV. Our study will provide novel insight into molecular basis of ASD and SCZ, and may lead to novel strategies to treat ASD and SCZ.

Loyal Goff, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Kabuki Syndrome 1

Single Cell Analysis of Hippocampal Neurogenesis Defects in Kabuki Syndrome 1

Intellectual disability is common, yet there are very few treatments available for this diverse group of individuals. Patients with Kabuki syndrome (KS) exhibit reduced intellectual capacity relative to their peers in the form of learning and memory defects. KS arises from a mutation in one of two genes that help to establish the epigenome, or 'regulatory' layer that governs when genes are turned on or off. 70% of KS patients exhibit mutations in one particular gene, KMT2D, that establishes a permissive environment to turn on gene expression at a wide variety of genomic loci.

To better understand how intellectual disabilities emerge in KS patients, we have characterized a mouse model with a similar mutation. KS-mice exhibited significant deficits in specific tasks and behaviors associated with learning and memory relative to normal mice. These tasks are associated with the proper functioning of a region of the brain known as the hippocampus; one of only a few regions in which neurons are produced throughout adulthood. The production of neurons in this region is significantly reduced in the mutant mice, however, it can be reversed by administering a drug that acts to enhance gene expression genome wide. This result has left us with an important question. How are new neurons specifically disrupted in a disorder caused by a mutation in a gene that is supposed to act across the entire genome? We hypothesize that there is a subset of genes, particularly sensitive to regulation by KMT2D, that are specifically misregulated in the hippocampus during neuron formation, and result in a failure to produce new neurons.

We are proposing to combine several cutting-edge techniques. We will 'reprogram' the cells from the skin of KS patients into cells with the capacity to become virtually any cell type in the body. We will take these disease-specific induced pluripotent stem cells (iPSC) and use them to make neurons in a dish. We can sample individual cells and measure the expression level of every gene at once during this process using a technique known as single-cell RNA-Sequencing. We can use these measurements to arrange the cells in an order that reflects their progress towards becoming a mature neuron. We hypothesize that iPSC with mutations in KMT2D will fail to become fully mature, and we will be able to identify the specific point in this process where this block occurs. We can identify the specific genes that are different at these key timepoints, and evaluate their involvement in neuron production and their potential as therapeutic targets.

We will treat the cells in a manner to alter the expression of these key genes and evaluate whether these manipulations rescue the production of mature neurons in these cells. We can confirm our results in the mutant mice. Using this approach we will identify the specific genes that are responsible for the reduction in adult neuron formation. Using this information we will evaluate various therapeutic options targeting these specific genes that may serve to improve the intellectual impairment associated with Kabuki syndrome.

Warren Grayson, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $690,000
Disease Target: Volumetric Muscle Loss

Engineering Contractile Muscle for Treatment of Volumetric Muscle Loss

Skeletal muscle has a high capacity to regenerate following minor strains and tears. However, when muscle damage results in gaps in the tissue greater than 20% of the entire tissue volume (e.g. in cases of severe automobile accidents, blast injuries, or tumor removal), the ability of muscle to spontaneously heal is overwhelmed. The outcome is volumetric muscle loss and it leads to a significant decrease in motor function and an impaired quality of life. Approximately 40% of all traumatic injuries involve skeletal muscle damage resulting in an annual global economic burden of roughly $6 billion.

The clinical gold standard of treatment remains the surgical transfer of muscle flaps from another region of the patient's body. This approach improves the aesthetics of the damaged anatomy but is associated with poor functional and regenerative outcomes. Consequently, tissue engineering approaches have the potential for significant impact in the field of skeletal muscle regeneration by combining stem cells with advanced biomaterials and providing instructive signals to create contractile muscle grafts that are responsive to physiological stimulation. Collaborative efforts among our labs have resulted in a promising strategy to engineer skeletal muscle grafts that can be transplanted and sutured in place to regenerate lost muscle tissue. Starting with the patient's own skin cells, we propose to generate large quantities of stem cells, place them on hydrogel scaffolds that have been engineered to mimic the structure and stiffness of native muscle, and 'exercise' the resulting tissues by providing physiologically relevant stretch in the presence of the appropriate nutrients and growth factors to stimulate new muscle growth.

We will test the feasibility and effectiveness of this approach by implanting the engineered grafts into mice in which two of the muscles on a single leg have been surgically removed. The genetically modified mice can tolerate human cells without immune rejection and provide a rigorous model to assess tissue regeneration. The grafts will remain in the mice for up to 3 months during which the mice will maintain normal activities with or without an additional hour of exercise per day. The proposed studies will specifically test the hypothesis that physical signals stimulate stem cells to regenerate functional skeletal muscle. This approach addresses key scientific and practical challenges and provides a promising therapy for VML treatment: (1) The stem cells can be derived from the patient's own tissues eliminating the need for immunosuppression. (2) Defined chemical supplements will be used to stimulate the differentiation into muscle cells, facilitating subsequent transplant into patients without the risk of disease transmission. (3) The advanced hydrogel scaffolds can be customized to the size of any muscle defect scaled up and bundled to. (4) The studies will provide novel information on the role of physical stimulation on the growth, differentiation, and integration of stem cells to regenerate skeletal muscle following volumetric muscle loss.

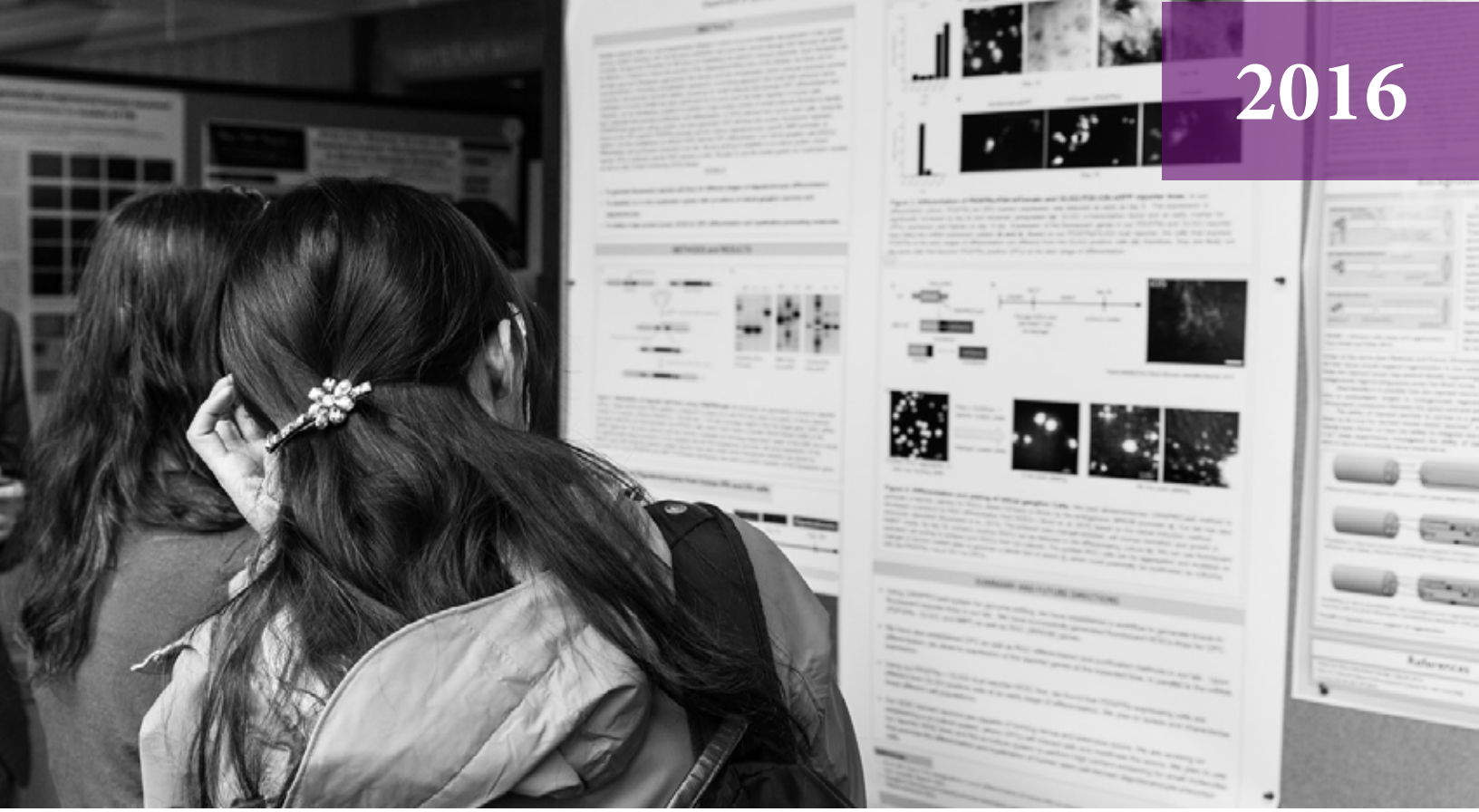
Gordon Tomaselli, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $682,842
Disease Target: Long QT syndrome, Brugada Syndrome, Myotonic Muscular Dystrophy

Tissue Models of Heritable Cardiac Arrhythmias

Sudden cardiac death (SCD) is a major public health problem with over 200,000 deaths annually in the United States. Rare inherited irregularities of the heart rhythm offer important insights into the sudden demise of patients with very common heart diseases such as heart failure and into uncommon but dangerous complications of a variety of medications (including routinely prescribed drugs such as antibiotics and antihistamines). The ability to create functioning heart cells from adult stem cells derived from blood opens up exciting opportunities to study disease and drug responses in human tissues that would otherwise be very difficult to obtain (for example heart and brain which would otherwise require biopsy). In this project we will study heart cells in 3D models that we have developed to simulate human heart muscle. We believe that placing heart cells derived from blood stem cells will create a more realistic model in which to study human heart diseases and the response of the heart to medications. This approach holds the promise of better understanding SCD and developing better methods to prevent this devastating condition and to better test medications before they are released for use in humans.

The primary goal of the MSCRF is to support research that will advance the field of regenerative medicine. In this project, we will focus on the most common heritable cause of ventricular arrhythmias, LQTS. This disease alters the electrophysiology of the cardiac cell, rendering heart muscle susceptible to rapid, potentially lethal electrical activity. Interestingly, acquired electrophysiological defects in heart cells that resemble congenital LQTS are common and seen in heart failure and in association with some drug therapies (acquired LQTS). The understanding of LQTS at the level of the heart muscle is of paramount importance to the development of appropriate genetic and pharmacological therapies to, at best reverse the disease and at least minimize the risk of the most devastating complication sudden cardiac death. Our work will generate complementary 3D tissue-based models of normal human muscle and LQTS (types 1-3) and generate cell lines that can accurately model disease processes in vitro. The 3D models will facilitate more robust risk assessment for SCD in patients with both congenital and acquired forms of LQTS and provide a higher fidelity assay for the cardiac safety of pharmaceuticals in both patients and control subjects.

These 3D models will also serve as platforms to test new therapeutic approaches (genetic and pharmacological) directed at correcting the electrophysiological defects of LQTS and related disorders in intact heart tissue and patients. Over the course of this 3-year proposal the utility of these platforms for drug testing to assess the liability of a compound to produce arrhythmias associated QT prolongation will be adjudicated. Several methods for correcting gene mutations in cells already exist and we will be able to develop protocols to apply these methods to intact tissue models and assess their efficacy. The cell and tissue platforms will also allow for assessing the therapeutic efficacy of genetically and pharmacologically manipulating channel regulators. This work will advance the field of regenerative medicine by creating tissue models of the heart that can be used to understand the pathogenesis of LQTS, assess the feasibility of genetic and genomic interventions to correct mutations in intact heart tissue and define new therapeutic targets for the treatment and prevention of sudden cardiac arrest. This work represents a significant advance toward the goal of personalized, precision medicine and therapeutics. The program will be an integral part of the bench to bedside approach that characterizes patient and disease management in the Center for Inherited Heart Disease at Johns Hopkins School of Medicine.



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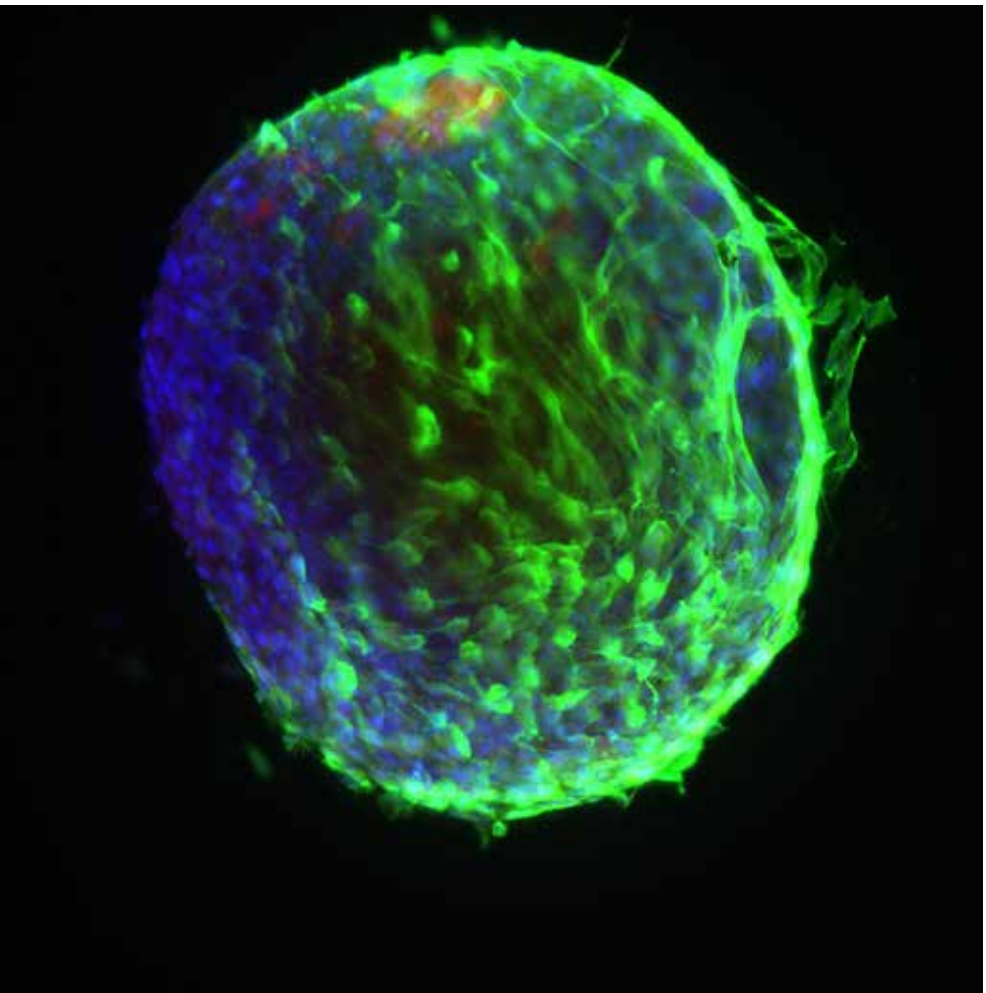


Cynthia Berlinicke, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Age Related Macular Degeneration (AMD)
Retinal Degeneration Retinitis Pigmentosa

Single Cell Transcriptomic Analysis of Stem Cell-Derived Retinal Cups

The retinal degenerative diseases (RD), which include retinitis pigmentosa (RP) and age related macular degeneration (AMD), are diseases that cause dysfunction and death of photoreceptor (PR) cells, resulting in vision loss and often leading to blindness. Cell transplantation is one proposed strategy that holds great promise, but clearly it would be more desirable and cost effective to block disease progression before transplantation becomes necessary. We therefore propose to study the process of retinal development and degeneration using human optic cups that are created in the laboratory from human induced pluritpotent stem cells (iPSC). More specifically, we propose to determine the changes in gene-expression associated with human retinal development and those that occur during the development of optic cups under conditions associated with retinal degeneration. The genetic pathways we identify to be important for these processes could potentially be targeted to help develop safe and effective drugs for the treatment of the blinding retinal degenerative diseases, most of which are currently untreatable. Human iPSC differentiated retinal cells are an invaluable tool not only for potential retinal replacement therapies, but also for understanding the etiology of, and developing treatments for, retinal diseases. In this proposal we are using in vitro differentiation of hiPSC into three-dimensional optic cups as a model for retinal development and degeneration. We will be doing a comprehensive analysis of changes in gene expression at the single-cell level through the course of these processes, a study that cannot be done except by using this unique system. The data from this analysis will reveal novel networks and pathways that are involved in photoreceptor development and degeneration and be will be publicly available through the Gene Expression Omnibus (GEO). Modulation of key factors in retinal development and degeneration pathways could provide lead targets for future therapeutic development.

Peter Calabresi, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Multiple Sclerosis (MS)

Function and Myelination of Human Retinal Ganglion Cells Bearing Polymorphisms Derived from the MS-A

Multiple sclerosis (MS) is a disabling neurological disease characterized by inflammation, loss of myelin and nerve cells of the brain and spinal cord. In this disease, immune cells attack cells in the brain, leading to loss of the protective myelin that coats the nerve projections called axons, which are necessary for nerve cells to communicate signals. It has a wide range of severity, from mild forms in which people can go about their daily lives, to severe forms, in which people become wheelchair-bound. Recent studies suggest that there is an inherent predisposition that leads people to develop more severe disease. Because the affected cells are primarily located in the brain, the ability to study this process has been hampered. We propose to combine stem cell technologies and results of genetic analyses to develop a new model system in which we can study the role of specific genes in the process of neurodegeneration and to investigate early events. We have identified genes that we think may be important for nerve degeneration by conducting an intensive genetics of disease severity study on people with MS, using a technique called optical coherence tomography (OCT), which measures the thickness of the nerves in the back of the eye, a part of the brain called the retina. Retinal thickness shows how severe the nerve damage is in patients, and is a method to track disease progression in patients. Using this technique, we evaluated a large number of patients (600) and studied patterns of each patient's DNA sequences and compared them to their measure of nerve damage over time as measured by OCT. We then identified a series of genes that correlated with the change in the thicknesses of the patients' retinas, and by extension, severity of the disease. We now hope to use novel stem cell cultures to study how these DNA variations lead the brain cells to become more susceptible to being killed, which then leads to disease. We will be able to use patient-specific cells to study these individual variations. Specifically, we will use peripheral blood cells of patients to generate inducible pluripotent stem cells (iPSCs), which we can then put into culture and coax into becoming a kind of nerve cell in the back of the eye called a retinal ganglion cell (RGC) by adding different factors. We will then have RGCs that naturally express the genetic variants in culture and from there, we can determine why they are more susceptible to being killed and ultimately, we can use these cells to test new therapeutic approaches to determine whether we can develop new drugs that will keep them alive. This work will provide new insights into the relationship between genetics, clinical progression, and functional loss in the brains of people with MS.

Amy DeZern, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Aplastic Anemia

Regenerative Medicine to Restore Normal Hematopoiesis in Aplastic Anemia

This is a high impact proposal using adult hematopoietic stem cells (HSCs) in an attempt to use regenerative medicine to cure the bone marrow failure (BMF) syndrome of aplastic anemia (AA). This disease is marked by ineffective hematopoiesis. Acquired AA affects thousands of younger and older patients worldwide each year. The disease consumes extensive resources and confers significant morbidity and mortality, due to their progressive nature and complications of suboptimal therapy. Without definitive treatment, mortality from severe AA approaches 70% at two years. Unfortunately, patients with acquired AA may have a response to immunosuppressive therapy (IST), but clonal evolution to myelodysplastic syndromes (MDS) or PNH develops in up to 40% of the patients, usually many months to years after the diagnosis of AA. Allogeneic BMT has the potential to cure AA and prevent the outgrowth of clonal hematopoiesis but is currently not offered as frontline therapy to patients who either lack a matched sibling donor or are perceived as too old (>40 years) to receive an upfront BMT. AA is a prime target for regenerative medicine because the current standard therapy of IST is not a cure and there are late results as above. HSCs derived from blood or marrow may also be used for regenerative medicine. Their major limitation for AA patients has been the inability for most patients to find a perfectly matched donor and the development of complications such as graft-versus-host disease (GVHD) and life-limiting infections after standard hematopoietic stem cell transplant (HSCT) regimens. We have developed a novel HSCT procedure that is so effective at eradicating GVHD and minimizing post-transplant infection and death that we no longer require matched sibling donors; half-matched donors are equally effective. Half-matched related donors are easy to find and make HSCT possible for all patients in need; any parent, any child and 50% of brothers and sisters or half-brothers and sisters will be half matched. The average person in the United States has 4.5 half-matched donors, while only 25-50% of patients in need of transplant have one perfectly matched donor (<20% for minority populations). One focus of this application is to refine the upfront BMT process to avoid toxicity and improve outcomes for all AA patients, regardless of available matched sibling donors. This involves expansion of the BMT donor pool through the use of alternative donors (haplo-identical) by reducing the complication of graft versus host disease (GVHD) with post-transplant cyclophosphamide. The second focus of this application is to identify the presence of somatic mutations in AA at varied times points in the disease. The literature suggests that clonal hematopoiesis is prevalent at diagnosis in AA but its correlation with disease phenotype and outcome is limited. The goal of this application is to optimize diagnosis and enhance therapy for AA. The ultimate objective is to apply these methods to all BMF disorders. We hypothesize that a novel approach to BMT using post-transplant cyclophosphamide may expand its availability and limit its toxicities in patients previously untreated for AA.

Tami Kingsbury, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Hematopoietic Disorders, Bone Marrow Transplants

Eyes Absent-1 (EYA1) as a Novel Hematopoietic Stem-Progenitor Cell Regulator

Hematopoietic stem cells can both self-renew themselves to maintain the hematopoietic stem cell pool and differentiate to produce all the blood and immune cell types required throughout an individual's lifetime. Hematopoietic stem cells are used clinically for life-saving bone marrow transplants, but their low abundance combined with our inability to produce substantially increased numbers of hematopoietic stem cells outside of the body greatly limits their utilization. Insufficient hematopoietic stem cell numbers are particularly acute in umbilical cord blood units, where an individual placenta (afterbirth) often fails to yield enough hematopoietic stem cells for clinical use or provides enough cells only for small pediatric patients. Improved methods to enhance hematopoietic stem cell self-renewal would significantly increase the number of patients able to receive bone marrow transplants as well as the types of new uses, such as gene therapy, that could be developed clinically.

Our genetic screening in the fruit fly model system has identified the protein "eyes absent" as an important molecule regulating hematopoietic stem cell proliferation and differentiation. When we experimentally decreased the amount of eyes absent in fly hematopoietic stem cells, the numbers of hematopoietic stem cells were reduced. Conversely, experimentally increasing the amount of the mammalian form of eyes absent was reported by others to potentially increase the numbers of mouse hematopoietic stem cells obtained in culture. Increased levels of eyes absent in other mammalian tissues such as kidney increased stem cell numbers, consistent with eyes absent playing a fundamental role in stem cells. These observations suggest that the human form of eyes absent may regulate human hematopoietic stem cells and thus provide a valuable tool to stimulate hematopoietic stem cell expansion outside the body. In this exploratory proposal, we will test the function of the mammalian eyes absent protein in human blood cell formation. The overall goal of this project is to determine new molecules that can be manipulated to increase expansion of human hematopoietic stem cells that will enable large-scale commercial generation of these cells for clinical transplantation and transfusion.

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

Human iPSC-Derived Extracellular Vesicles: Targets for Novel Diagnostic & Therapeutic Strategies

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, nor are the earliest times to intervene to prevent or reverse the course of the disease. Our application is based upon recent successes in our laboratory generating human induced pluripotent stem cells (hiPSCs) by reprogramming skin cells from both patients with early onset AD (ADhiPSCs) and healthy individuals (WTiPSCs) into cells similar to embryonic stem cells and differentiating them further into human brain cells that display important disease properties. These cells secrete small (50-1000nm in size), membrane-bounded vesicles, called exosomes and micro-vesicles (collectively EMVs) that carry proteins, RNAs and metabolites. Extracellular vesicles can be also found in all the biological fluids, such as blood. Most importantly, we have recently shown that EMVs secreted from our AD iPSC-derived neuronal cultures, as well as from blood of AD patients able to transfer AD pathologic agents that could play a role in the formation of amyloid plaques and neuronal damage. Our research investigates the potential application of the EMVs secreted from hiPSCs and their neuronal progeny in developing biomarkers for the early diagnosis of AD, understanding disease propagation and reversing AD pathology. To achieve this goal, we have assembled a remarkable team of experts on AD and aging, and pioneers in the discovery of novel biomarkers from AD patients's blood for early diagnosis of the disease. We will work together to purify and extensively characterize the AD-related protein composition of EMVs from ADiPSC-derived neuronal cultures and control subjects. We will further use these EMVs to validate already proposed AD biomarkers or discover new ones. In this application, we propose also to build compelling in vitro models of AD from human iPSC cells, defined here as cortical organoids that mimic the multi-cellular composition and three-dimensional (3D) organization of brain tissue. We propose that introducing EMVs from ADiPSC-neuronal cultures or from AD patient's samples to our systems, will maximize the chance that our model will generate Aβ plaques or pre-plaques because this 3D environment is key to reproducing real brain conditions and allowing Aβ plaques to form and remain stable. More importantly, for the first time we will be able to study the propagation of the disease in a model that resembles the human brain.Finally, based on recent exciting data and the expertise of our co-Investigators, we will begin to study the potential role of hiPSC-derived EMVS as vehicles of therapeutic molecules capable of reversing the AD pathology and neuronal damage. We envision that our studies will substantially increase the level of knowledge of the molecular mechanisms of AD pathogenesis and speed up drug development.

Cyrus Mintz, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Neonatal Hypoxic-Ischemic Brain Injury

Stem Cell Therapy for Neonatal Hypoxic-Ischemic Brain Injury

The proposed project is designed to test the potential of human neural stem cells, derived from skin cells rather than embryonic sources, to treat neonatal hypoxic-ischemic brain injury (HI). This disorder occurs when a newborn suffers a loss of oxygen delivery to the brain, typically due to a mishap in the birthing process such as umbilical cord strangulation, or due to complications of congenital heart disease or lung disease associated with prematurity. It is an unfortunately common condition: nearly one million newborns each year suffer from neonatal HI worldwide. The consequences range from moderate neurologic impairment to paralysis and severe mental retardation, and burden both in terms of human suffering and monetary costs for society are enormous. The essential cause of disease in neonatal HI is the loss of neurons due to cell death resulting from inadequate oxygen delivery to the brain. The only therapy that currently exists is hypothermia, which may not be practical to institute for all patients and is only moderately protective in any case. Therefore, in this project we will test the capacity of a type of neural stem cell, known as a human neural progenitor cells (hNPC) to survive transplantation into a the brain of a mouse that has been injured using a commonly accepted approach to mimic neonatal HI. The hNPCs could be derived from the patient or family members, and thus would avoid issues of immune rejection as well as ethical and practical issues related to the use of embryonic tissue. Furthermore, cells at a similar stage exist in the developing brain, and thus the hNPCs are likely to be readily incorporated into functioning neural circuits. We will determine how well the transplanted hNPCs incorporate into the injured mouse brain and also test for enhanced recovery due to the stem cell transplant. Additionally, we will test two promising drugs, neurotrope J147 and erythropoeitin, for their capacity to support hNPCs transplants. First we will determine the extent to which these drugs can support the growth of similar neural stem cells that reside in the mouse brain, and then using this information we will determine whether they can further support and enhance hNPC transplants. This project is designed to test a cutting edge stem cell therapy for its potential to treat a devastating human disease and it has the potential to lead to rapid translation to a human therapy.

Enid Neptune, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $230,000
Disease Target: Bronchitis, Asthma, Toxic Airway Injury

Developing Stem Cell Therapy for Airway Disorders

The primary goal of this proposal is to develop innovative stem cell therapy for the debilitating lung disease that affects millions worldwide: chronic obstructive pulmonary disease (COPD). COPD is a comprised of a group of progressive, debilitating lung conditions that include emphysema and chronic bronchitis primarily caused by exposure to cigarette smoke or environmental toxins. COPD became the third leading cause of death in the United States as early as 2008. Moreover, one in 20 deaths in the US is secondary to COPD. Severe asthma with COPD is caused by various insults, including exposure to environmental toxins or cigarette smoke. COPD can also result from progressive damage to the airways with airway blockage from asthma. Even normal aging is associated with changes in lung tissue that can lead to COPD. All of these factors cause structural and functional loss of distal lung tissues, which in turn, compromises oxygen delivery and lung function in COPD. Despite the substantial disease burden, there are no effective therapies to interrupt the process or repair damaged lung tissue, excluding lung transplantation. However, lung transplantation is not a practical therapy for most people with COPD due to the significant morbidity associated with this highly invasive procedure, the lack of available donors, and the high cost. Unfortunately, therapy is therefore largely supportive and limited to supplemental oxygen, anti-inflammatory agents, or asthma therapy such as bronchodilators. Importantly, our population of aging adults is growing and the incidence of COPD is also rising. These stark epidemiologic facts underscore the urgent need for better therapies for COPD. Here, we propose to develop innovative stem cell therapy in which we derive lung stem cells for use in regenerating lung tissue to treat patients with COPD. Early interventions that arrest or reverse the development of COPD will not only reduce morbidity and mortality, but also will decrease the incidence of chronic, debilitating lung disease. We discovered that we can convert lung cells into stem-like cells that grow and expand outside of the lung and can be converted back into mature lung cells. We plan to use this technology to generate lung cells that could be used to regenerate healthy airway tissues for patients with disorders like COPD.

Giorgio Raimondi, Ph.D.
Johns Hopkins University, School of Medicine
Award Amount: $300,000
Disease Target:Transplant (Tissues and Organs) Rejection

Engineering a "Hybrid Thymus" to Promote Transplant Tolerance & Graft-Protective Immunity

The successful use of tissue and organ transplantation to save patients with end-stage diseases is hampered by the shortage of tissues and organs available for donation and by the unresolved issue of preventing rejection without the use of toxic immunosuppressants. Regenerative medicine is at the forefront of solving the problem of tissue and organ shortage via phenomenal advancements in stem cell research and tissue engineering. However, stem cell-derived tissues will still be subjected to a rejection response by the patient's immune system. There is then an urgent need for a therapeutic protocol that induces lasting tolerance. We believe that the synergistic work of transplant immunology and stem cell research holds the key for this solution.

The thymus has a fundamental role in the establishment of immune tolerance. Within the thymus of healthy individuals, lymphocytes are trained to accept and defend all tissues of the body. Given this fundamental role of thymic cells in establishing self-tolerance, we propose a novel strategy for promotion of transplant acceptance: the creation of a donor (stem cell line)-recipient "hybrid thymus". Specifically, we aim to re-engineer the thymic architecture via injection of new thymic cells derived from stem cell lines to create a hybrid environment where developing immune cells are trained to accept both patient and stem cell-derived tissues. Our hypothesis is that via this procedure we will change the patient's central tolerance process favoring the establishment of immune tolerance toward a tissue (or organ) engineered from the same stem cells, ultimately promoting lifelong acceptance without the need for chronic immunosuppression.

If successful, this exploratory project will pave the way for a strategy that could then develop into the treatment of choice for induction of indefinite survival of stem cell-derived tissue and organ transplants while preserving the complete functionality of the patient's immune system (differently from currently investigated tolerance inducing protocols). This strategy would be characterized by its simplicity and efficacy; a major breakthrough in transplant research and regenerative medicine that will accelerate the development and delivery of therapies to patients, improving the likelihood of success and providing new hope for thousands of people.

Chinmoy Sarkar, Ph.D.
University of Maryland, Baltimore
Award Amount: $230,000
Disease Target: Traumatic Brain Injury

Neuronal Differentiation of iPS Cells by Autophagy Induction in Oxidative Environment to Treat TBI

Traumatic brain injury is one of the major causes of death and neurological impairment among young adults. As per Centers for Disease Control and Prevention around 2.5 million cases of TBI have been reported annually in the United States1. Primary causes may include motor vehicle accident, falls, assaults and sport related injury among civilian population and blast injury among military personnel in active duty at the combat zone. TBI accounts for almost 30% of injury related death. Patients also suffer from lifelong neurological disability, which not only causes enormous physical and emotional distress to them and their families but also leads to huge economic burden to the affected individual and the society. Moreover TBI is considered as one of the major risk factors for other neurodegenerative disorders like, Parkinson‘s and Alzheimer‘s diseases.

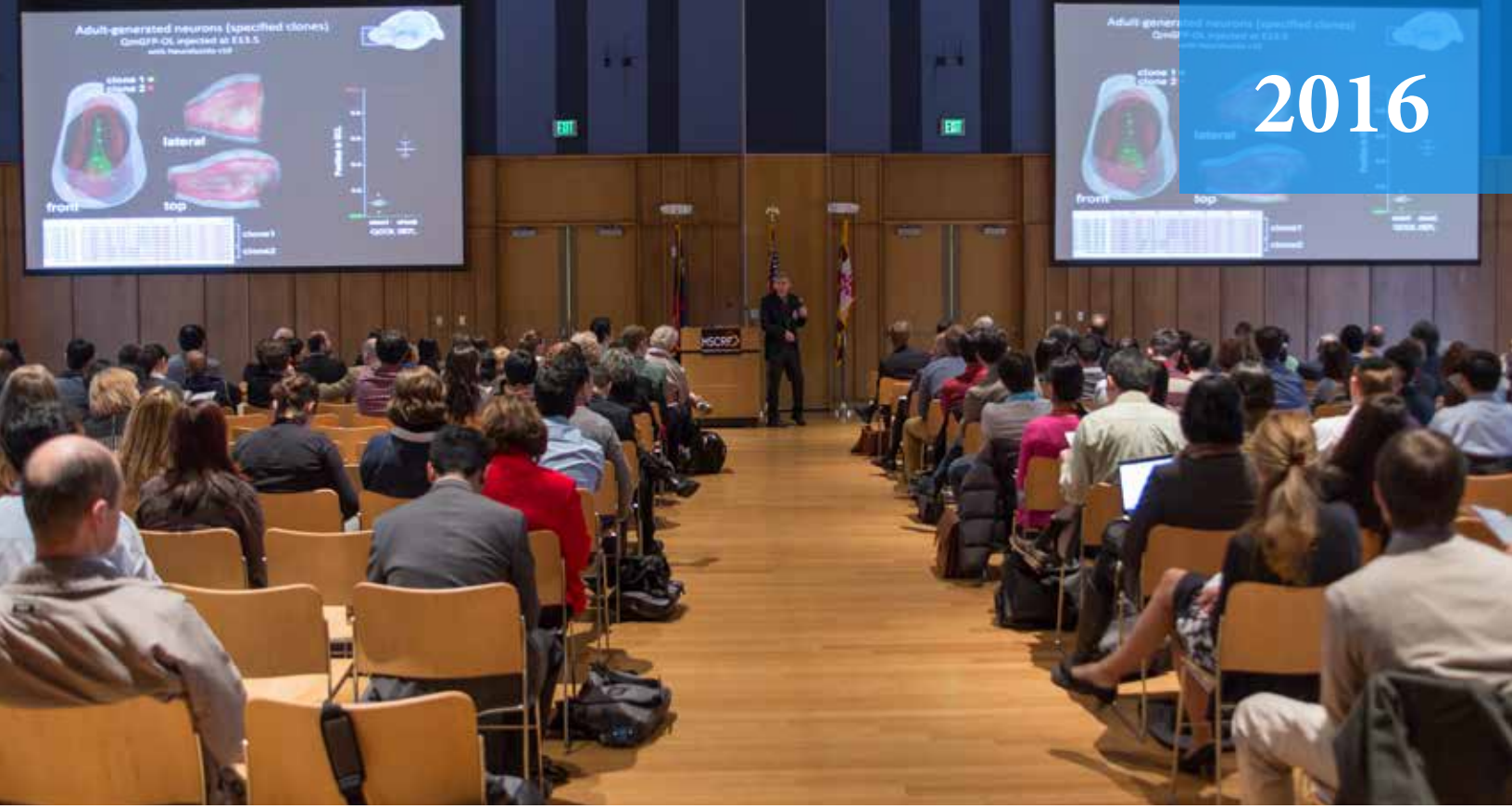
TBI associated neurological impairment is caused by severe and progressive neuronal cell death. Since neurons unlike most other cell types are unable to divide, body‘s own repair system cannot replace neurons lost after TBI. Thus implantation of neural stem cells (NSC) at the injury site is most viable therapeutic approach to replenish the lost neurons in TBI patients. However, the environment at the injury zone of the brain of TBI patients is highly oxidative which is detrimental for neural stem cell survival and proper differentiation into neurons2. Unfortunately, to date no pharmacological agent has been found to be effective in reducing oxidative damage in patients with moderate and severe brain injury thereby limiting the potential benefits of NSC implantation in TBI.

Autophagy is a cellular degradative process, which clears damaged toxic materials from the cells. It also plays crucial role in neuronal stem cell survival and differentiation. We recently demonstrated that autophagy is inhibited after TBI, contributing to neuronal cell death. Therefore, in addition to being highly oxidative, the injury site is also not conducive for autophagy necessary for NSC survival and differentiation. We propose that re-activation of autophagy could protect NSCs under oxidative environment of TBI and promote their proper differentiation into neurons. Thus in accordance with the goal of MSCRF to develop new treatments for human diseases through human stem cell research we will use human iPS cell derived NSCs (iPS-NSC) to evaluate autophagy activation as means of protecting NSCs and promoting their differentiation into neuron under oxidative conditions. We will also explore the therapeutic potential of human iPS-NSCs with activated autophagy in TBI by transplanting them into the brains of mice after TBI.

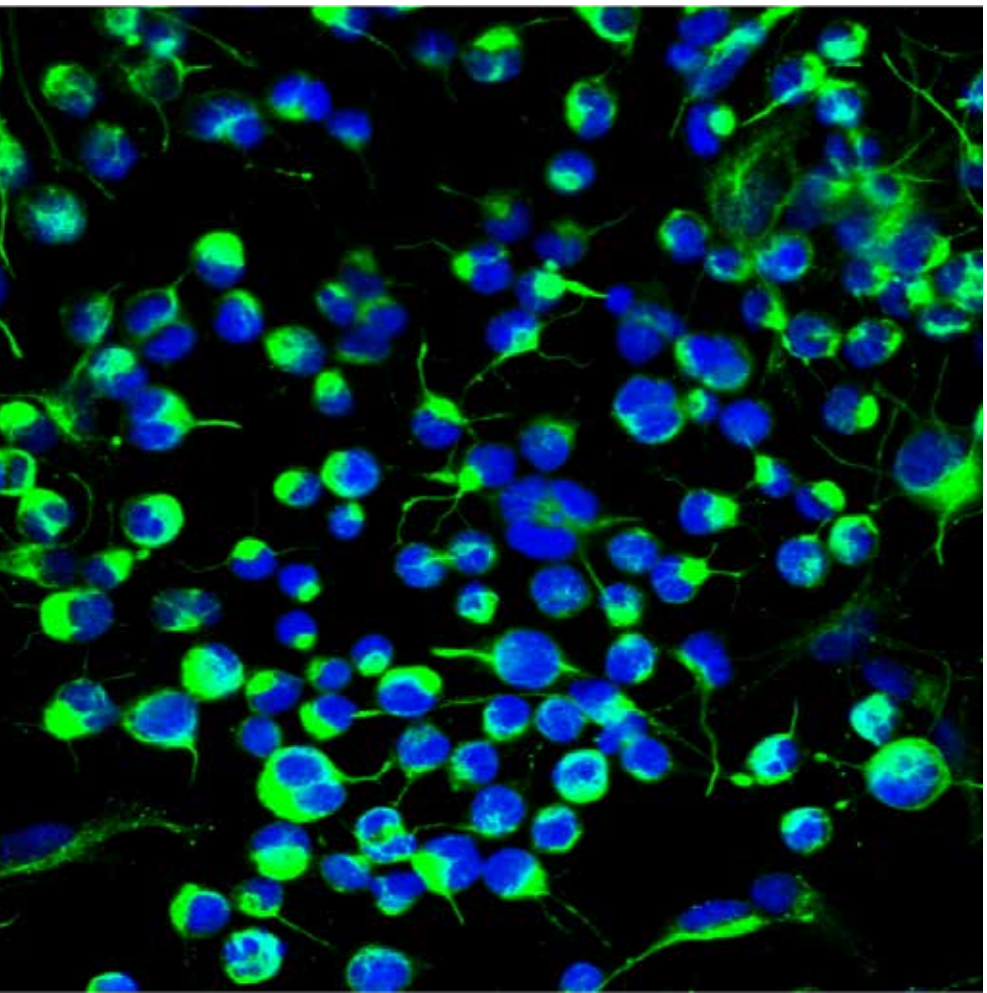
Kathryn Wagner, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
Award Amount: $111,875
Disease Target: Muscular Dystrophy, Myopathy

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

Stem cell therapy is considered by many to hold the promise of a cure for chronic muscle disorders such as the muscular dystrophies. However, when stem cells are transplanted into dystrophic muscle, they do not typically survive nor form new muscle. Our hypothesis is that stem cells must begin engraftment in a more hospitable environment. We have developed an injectable biosynthetic scaffold for muscle progenitor cells derived from human induced pluripotent stem cells. These cells form contractile myofibers. We now propose to optimize and test this system in an animal model of muscular dystrophy. The translational plan for this work is to provide proof-of-concept of the benefits of hiPSC-derived myoblasts in a disease model with the studies described in this application followed by optimization of conditions compliant with Good Manufacturing Practice. We have already had multiple discussions with the JHU Cellular Processing and Gene Therapy Facility (CPGT) which will provide us with QA/QC and regulatory guidance as we prepare for the next step which would be manufacture of our iPSC-derived myoblasts and hydrogel under strict GMP protocols. We are fortunate that the myostatin inhibitor that we are working with has entered clinical trials (Pfizer 06252616) and may be granted separate FDA approval. I hold an IND for PF-06252616 in another indication and am the chairperson of the international Therapeutic Advisory Committee of TREAT-NMD. I have expertise in the types of IND-enabling studies that will be necessary to bring a product such as this to clinical trial in muscular dystrophy.



Post-Doctoral Fellowship Grant Awards:



Post-Doctoral Fellowship:

Allison Bond, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Guo-li Ming, Ph.D.
Award Amount: $110,000
Disease Target: Schizophrenia, Depressive Disorders

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

Schizophrenia is a neuropsychiatric disorder that affects 1% of the world's population and is characterized by aberrant perceptions, social withdrawal, and deficits in attention and working memory [1]. While the pathophysiology is poorly understood, postmortem studies and genetic studies have provided consistent evidence that alterations in the GABAergic transmission plays an important role in both schizophrenia and major depressive disorders [2]. Disrupted-in-schizophrenia-1 (DISC1) is a genetic risk factor for schizophrenia and other affective disorders, and disruptive mutations in this gene have been used to model psychiatric disease [3, 4]. Here I will generate GABAergic interneurons from induced pluripotent stem cells (iPSCs) derived from controls and from patients with a 4 base-pair frameshift deletion at the DISC1 carboxy (C) terminus so that I can compare and contrast the transcriptomes of specific human GABAergic interneuron subtypes and determine the effect of DISC1 mutations on these transcriptomes.

Specific Aim 1: To compare the transcriptomes of GABAergic interneuron subtypes derived from human iPSCs
Specific Aim 2: To determine whether a mutation in DISC1 differentially affects the transcriptomic signature of human GABAergic interneuron subtypes.

The proposed project will determine whether the disease-associated risk factor, DISC1, differentially affects the molecular signature of specific interneuron subtypes. This study will provide important new insights into the relationship among psychiatric disease, genetic risk factors, and GABAergic system dysfuction.

Rebecca Fawcett, Ph.D.
University of Maryland, Baltimore
Mentor: Steven Bernstein, Ph.D.
Award Amount: $110,000
Disease Target: Glaucoma

Analysis of the Newly Identified Human ONL Stem Cells and their role in Glaucoma

This project will transform current treatment of the age-related disease primary open angle glaucoma (POAG). POAG is the second most common cause of blindness in African-Americans after diabetes. POAG severity also typically progresses despite the current treatment regime: Pressure-lowering eye drops(1-3). This fact compels the development of new strategies. The Bernstein lab has discovered that the human optic nerve lamina (ONL), a small transitional region between the eye and optic nerve, is an adult neural stem cell/neural progenitor cell ANSC/NPC) niche responsible for maintaining normal regulatory functions between the eye and nerve. My work will translate this discovery by demonstrating that ONL-NPC deficiency likely contributes to the severity and progression of glaucoma-related damage in rodents and humans, and by further identifying the ONL-NPC's previously unrecognized growth factors that support eye function. This work promises to provide a new, previously unknown approach to glaucoma treatment, which will improve outcomes for glaucoma.

This project is innovative in at least two critical areas: 1) Characterization of the glaucomaneuroprotective functions of ONL-NPCs in a tissue previously unsuspected of having these cells. 2) Generating a new approach to glaucoma therapy, using ONL-ANSC/NPCs and/or growth factors derived from the human ONL cells as a 'cocktail'. If successful this approach will greatly improve current glaucoma treatment.

Primary Open angle Glaucoma (POAG) pathophysiological mechanisms are poorly understood and only partially treatable, resulting in many cases of blindness. Study of the effects of adult human ONL ANSC/NPCs will enable further identification of the role of these factors in maintaining human RGC health, and the neuroprotective effects of their secreted factors in glaucoma and possibly other blinding diseases. The use of ONL-stem cell secreted factors, rather than direct stem cell transplantation, would be a novel adjunct clinical glaucoma treatment. This project thus has a high translational potential.

Miguel Flores-Bellver, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Maria Valeria Canto-Soler, Ph.D.
Award Amount: $110,000
Disease Target: Age-related Macular Degeneration

3D Neural Retinal/RPE Complex from Human iPS cells: a Novel Age-related Macular Degeneration System

Age-related Macular Degeneration (AMD) ranks third as a cause of blindness (after cataract and glaucoma) in the world, and is the most common cause of blindness in the United States 1. Unfortunately, AMD remains incurable. A critical limiting factor preventing effective treatments for the initial stages of AMD is our poor understanding of its early pathophysiologic events, and the lack of appropriate models for its study. Induced pluripotent stem cells (hiPSCs) provide unprecedented opportunities for the development of human cell-based models to study disease mechanisms and identify potential therapeutic agents. An in vitro "eyecup" derived from hiPSCs composed of all of the cellular elements of the neural retina and retinal pigment epithelium (RPE) could help to rapidly advance our understanding of the etiology of AMD. Thus, the objective of this proposal is to develop a muchneeded hiPSC-based 3D retinal model suitable for uncovering relevant physiological mechanisms associated with early AMD. This application is technology-driven in nature rather than being a classic, mechanistic hypothesis-driven proposal. MD is a complex, multifactorial disease, involving genetic and environmental factors2. The early stages of AMD are characterized by impaired physiological function of the RPE, which in turn leads to the death of rod photoreceptor cells within the parafoveal region of the macula3,4. Thus, cellular interactions between photoreceptor cells and the underlying RPE are at the center of the mechanisms that trigger AMD, but the specific nature of these interactions remains unclear3,4.Current animal models of AMD do not adequately recreate the features of this disease in the human retina5,6. On the other hand, the potential of human pluripotent stem cells to more closely mimic the pathophysiological aspects of this condition has led to the establishment of several stem cell-based models for AMD7-9. These models, while certainly aiding to identify mechanisms involved in AMD, still fall short in recreating complex physiologically relevant conditions since they consist primarily of twodimensional cultures of a single disease-relevant cell type, most typically the RPE7,8. Thus, our goal is to develop the first human mini-retina model consisting of a laminated neural retina (NR) and associated RPE derived from human iPS cells and capable of recreating the physical and functional interactions occurring between photoreceptors and RPE during the early stages of AMD.Our system would signify an important breakthrough because it would provide an innovative, highly versatile model to study the cellular interactions between photoreceptors and RPE involved in the early stages of AMD, with the precision of controlled in vitro conditions and the cellular complexity of the native retina. The innovative aspects of this model would: i) enable unique studies of interactions between photoreceptors and RPE that are not feasible in animal or other cell culture models; ii) enable careful studies delineating the specific role of genes and risk SNPs in AMD pathogenesis; iii) provide a platform for "personalized medicine" whereby iPS cells from individuals afflicted with AMD or another retinal disease could be studied or novel therapeutic agents tested.

Ziyuan Guo, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Hongjun Song, Ph.D.
Award Amount: $110,000
Disease Target: Neurofibromatosis Type 1 (NF1)

Investigating Cellular Mechanisms Underlying NF1-Associated Cognitive Impairments using iPSCs

Neurofibromatosis type 1(NF1) is monogenic disorder arising from either an inherited or de novo autosomal dominant heterozygous mutation in the NF1 gene [1]. Although tumor formation is the most prominent symptom associated with NF1, up to 80% of children with NF1 also suffer from some form of cognitive impairment and exhibit variable neuropsychological symptomatology [2]. Learning and attentional deficits in NF1 patients can have a profound effect on quality of life, but are among the most difficult impairments to diagnose and treat effectively. It has been shown that NF1 deletions contribute to abnormal synaptic function in drosophila and mouse model systems, however, the underlying mechanisms of human NF1 mutations at the cellular and neural systems level remain to be elucidated [3, 4]. Recent clinical trials using simvastatin, the closest approved alternative of lovastatin and a potent inhibitor of NF1-mediated Ras pathway, to treat NF1 children, have not successfully rescued their learning and attention deficits, suggesting a more complex role of NF1 mutations in human brains [5-7]. Patient derived iPSCs can serve as a unique cellular model for understanding the molecular and cellular mechanisms underlying tissue development and for deciphering disease-relevant pathological developmental events. In our lab, we have already generated several lines of iPSCs from NF1 patients. I will investigate the functional role of NF1 mutations in disease-relevant neurons. These studies could pave the way for the identification of cellular targets to develop new treatments for NF1 patients with cognitive impairments.

Aim 1: To differentiate NF1-specific iPSCs into forebrain cortical neurons and characterize synaptic function of neurons with NF1 mutations

Aim 2: To generate whole genome transcriptomes via RNA-seq to determine key dysregulated genes by NF1 mutations in cortical neurons

I will generate transcriptomic datasets in NF1-deficient and control human neurons to identify dysregulated genes in disease condition. These results will help to understand how disease causing mutations affect global gene expression within affected cells and to identify novel cellular target for the therapeutic treatment.

Hyunhee Kim, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Valina Dawson, Ph.D.
Award Amount: $110,000
Disease Target: Parkinson's Disease

Parthanatos in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting the lives of 1% of population who are 60 years old and older. The physical characteristics of the disease includes resting tremor, bradykinesia, and rigidity. It creates significant impediment on the daily life of affected individuals, thus, it is pertinent to find an effective strategy to treat the patients. The etiology of PD is understood as progressive cell death of dopaminergic neurons in the substantia nigria (1). So far no treatment is available to prevent the underlying cause; only some remedies to alleviate the symptoms (2, 3). This lack of "causative therapy" is primarily due to inadequate understanding of intracellular pathways responsible for progressive neuronal loss. Substantial research suggests that one of programmed cell death pathways responsible for this progressive neuronal death may be parthanatos (Fig 1). Poly (ADPribose) polymerase-1 (PARP-1) overactivation, which is a molecular marker of parthanatos activation, is found in mice with PD-like symptoms, while PARP-1 deficient mice undergo significantly less neurodegeneration after induction of PD (4). PARP inhibitors were shown to protect neurons against cytotoxicity induced by α-synuclein and 1-methyl-4-phenylpyridinium (MPP+) (5). We also recently found that AIMP2, whose accumulation in the substantia nigria contributes to neuronal loss, is a direct PARP activator (6). Therefore, it is evident that parthanatos by PARP-1 overactivation is a critical intracellular event leading to PD in animal models. Yet, we do not know if PARP activation also plays a significant role in human PD. In this study, we will examine the role of PARP in human sporadic and genetic models of PD, and test the efficacy of various PARP inhibitors in reducing cell death in PD neurons.

This project will use many latest technologies to introduce a number of important new concepts that have scientific merits in PD research.

- We will generate idiopathic human PD dopaminergic neurons using ESC differentiation followed by α-synuclein preformed fibril treatment
- We will employ CRISPR/Cas9 technology to generate PD neurons with Parkin deletion.
- This is a meaningful advance in the field of PD research, as it will be the first drug that targets specific intracellular signaling related to neurodegeneration. Other cytoprotective drugs under investigation, such as antioxidants and neurotrophic factors, have no specific molecular targets.

Our study has immense translational potential, as it may provide a strong rationale to further PARP inhibitors in PD clinical trials. PARP had shown to play a critical role in various cancer cell survival, so clinical efficacy and safety of PARP inhibitors had been intensively studied in the field of oncology (3). As the result, the safety of PARP inhibitors in this study was confirmed in numerous clinical trials. Also their therapeutic dose range, route of administration and pharmacokinetics are all well-documented, thus designing and implementing these drugs in PD clinical trials would not be difficult.

Srinivasa Rao Sripathi, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Donald J. Zack, Ph.D.
Award Amount: $110,000
Disease Target: Age-Related Macular Degeneration (AMD)

Modulation of RPE Epithelial Mesenchymal Transition as an Approach for Treatment of AMD

Age related macular degeneration (AMD) is a leading cause of vision impairment among the elderly worldwide. Currently, there are about 1.75 million Americans that have been diagnosed with some form of AMD. With the increased aging of the US population, the number of people with AMD is estimated to increase to 3 million by 2020 (Friedman et al., 2004). The progressive dysfunction and degeneration of retinal pigment epithelium (RPE), a monolayer of cells located on Bruch's membrane between the neural retina and the choriocapillaries, is thought to play a key role in the pathogenesis of AMD. Since RPE cells play such a key role in maintaining the health and function of photoreceptor cells, and are important in AMD pathogenesis, considerable attention has been devoted for studying their biology and pathology. Such studies were limited in the past to the study of animal models, human fetal samples, and cadaver and donor specimens. The recent advent of efficient methods for differentiating human ES and iPS into "RPE" (hRPE) cells that closely mimic endogenous RPE cells at the molecular, biochemical, morphological, and unctional levels has made possible important advances in our understanding of the biology and pathology of human RPE. The availability of hRPE cells used in conjunction with CRISPR/Cas9 genome editing technology has allowed the development of "disease in a dish" models, and also enables more sophisticated drug screening paradigms based upon high content analysis of hRPE cells. Additionally, the availability of hRPE cells has led to the initiation of several clinical trials to treat AMD patients by RPE cell transplantation (Schwartz et al 2012, 2015). However, despite the excitement of these ongoing clinical trials, many challenges are remain elusive. Some of which, relate to maintaining the differentiated state of the stem cell -derived RPE cells. A variety of even mild perturbations can induce dedifferentiation and an epithelial to mesenchymal transition (EMT) -like phenotype. Additionally, there is evidence that EMT may be involved in AMD pathogenesis in vivo. RPE cells lose their tight junctional integrity and acquire fibroblastic morphology during the progression of choroidal neo -vascularization (Lopez PF et. al 1996, Bailey TA et. al 2004). EMT has also been implicated in other retinal diseases such as the scarring associated with diabetic retinopathy and in proliferative vitro -retinopathy (PVR) that can be associated with retinal detachment (Tamiya et al 2016_ Amin R et al1994). As described below, over the past year I have developed an hRPE cell -based system for studying RPE EMT, and in this MSCRF application I propose to use this system to better study the mechanism of RPE EMT and to screen for small molecules that inhibit EMT. Such studies, I suggest, will provide useful new information on a molecular process that is important for AMD pathogenesis and treatment, and could also lead to the identification of potential lead molecules for the future development of novel therapeutics for AMD and other forms of retinal disease.

Dhruv Vig, Ph.D.
Johns Hopkins University
Mentor: Sean Sun, Ph.D. & Sharon Gerecht, Ph.D.
Award Amount: $110,000
Disease Target: Vascular Diseases

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

The capacity for stem cells to self-renew and to differentiate into other cell types makes them an essential component in embryonic development, tissue regeneration, and replenishing lost cells due to programmed cell death (apoptosis) (1). To sustain this function throughout an organism's lifetime requires a delicate balance of a stem cell's cellular potency (i.e. the cell's ability to differentiate into other cell types) and ability to self-renew (1). Proper regulation of stem cells along with other progenitor cells is required for tissue development and homeostasis, as occurs during the formation of blood vessels, a process known as vasculogenesis (2, 3). However, failure to maintain this homeostasis can result in life-threatening pathologies, such as hardening of the arteries (atherosclerosis) (4, 5) or cancer, which occurs when uncontrolled cellular proliferation causes tumor formation (1). A stem cell's fate is controlled by extracellular cues from its niche and by intrinsic genetic programs within the cell (1). Niches are spatially-distinct, heterogeneous microenvironments that include neighboring cells and extracellular material (6). Continuous crosstalk between stem cells and their niche has been shown to regulate stem cell behavior (7-11). One mechanism that permits crosstalk between stem cells and their environment is mechanotransduction, which occurs when cells respond to their environment by sensing external forces (12). Notably, previous works have used micro-pattern technology to geometrically confine human pluripotent stem cells (hPSCs) and found that circular confinement alters cell spreading, the formation of tensile actomyosin structures, stress fibers and actin bundles (13). In addition, geometric control of cytoskeletal tension has been shown to regulate endothelial cell survival (14), YAP/TAZ transcriptional activity (15, 16) and differentiation potential (17). In this proposal, we seek to quantify the effects of physical confinement and cell-cell spatial/mechanical interaction on hPSCs and begin to address how these effects drive global changes in tissue architecture and morphogenesis as hPSC differentiate into vascular cells. We hypothesize that biophysical signaling and geometry are an important factors in a stem cell's decision making process. Specifically, we first hypothesize that matrix-mediated cell-cell interactions (i.e., when cellular differentiation alters the strain in the matrix thereby influencing the forces that are exerted on nearby undifferentiated cells) is an additional biophysical mechanism that directs stem cell fate and second we hypothesize that geometric confinement presents the cells with a spatially varying mechanical stress that coordinates with chemical signals to influence differentiation efficacy. In order to test these hypotheses, we will explore how alterations in matrix elasticity, cell-cell contact and geometric shape and size affect the biophysics and differentiation rates of hPSCs. We will also develop a quantitative model that predicts how these differentiation rates and biophysics drive the spatial patterns observed when stem cells are confined (18-21). Our research will specifically address the role of geometry in cellular differentiation, while simultaneously addressing how various extracellular and biophysical mechanisms lead to the spatially distant patterns observed during development. Disentangling the biophysical cues that control hPSC differentiation is the first step towards establishing a novel differentiation protocol based on mechanical signaling .

Qingfeng Wu, Ph.D.
Johns Hopkins University, School of Medicine
Mentor: Hongjun Song, Ph.D.
Award Amount: $110,000
Disease Target: Psychiatric Disorders, Cardiovascular Diseases

Using microorganoids to Model the Effects of Maternal Stress on the Hypothalamic-Pituitary System

The lifelong health of an individual is shaped during critical periods of early embryonic development. Cumulative evidence from human studies suggests that maternal stress leads to prenatal overexposure to glucocorticoid hormones, which predisposes the fetus to postnatal challenges such as metabolic, cardiovascular diseases and mood and behavioral diseases, including major depression, schizophrenia and ADHD [1, 2]. Glucocorticoid hormones act at multiple levels within the embryonic brain, particularly the hypothalamic-pituitary-adrenal (HPA) axis, which is critical for maintaining systemic homeostasis by regulating hormone release [3]. Functional changes in HPA activity after prenatal exposure to glucocorticoids appear to be transgenerational and have long-term adverse consequences on the behavior of offspring [4]. However, it remains unclear how glucocorticoid overexposure impacts the cytoarchitectonic organization of brain areas involved in the HPA-axis and results in dysfunction of the hypothalamic-pituitary system. Recent development of three dimensional (3D) cultures, such as cerebral organoids, derived from embryonic or induced pluripotent stem cells (iPSCs) provides a new platform that more closely recapitulates the endogenous formation and development of different neural structures [5] and can serve as a better model to investigate the earliest events in human fetal brain development. I will develop and characterize hypothalamic organoids and use this micro-organoid model to test the effect of glucocorticoid overexposure on fate specification, structural organization and functional outcomes of the developing hypothalamic-pituitary system.

Specific Aim 1. To generate a model of hypothalamic and pituitary tissue development in three-dimensional (3D) microorganoids from human iPSCs.

Specific Aim 2. To investigate the effect of glucocorticoids on the organization and function of hypothalamic microorganoids.

Compelling epidemiological evidence has shown that prenatal exposure to intrauterine environmental insult is associated with metabolic and behavioral disorders in adulthood. As a consequence of maternal stress or treatment with synthetic glucocorticoids for various diseases during the pregnancy, fetal overexposure to glucocorticoids may exert long-term epigenetic programming changes of the HPA axis and result in impaired fetal growth, altered behavior and chronic disease. It is hypothesized that prenatal overexposure to glucocorticoids leads to aberrant patterning of brain areas and consequently their function within the HPA axis during fetal development [6]. However, there is no experimental evidence so far. The proposed research will generate novel cellular and molecular insights into the mechanisms underlying how prenatal glucocorticoid exposure modulates the structure and function of the developing hypothalamic-pituitary system. Furthermore, the 3D microorganoid model we generated here can be used to as a platform to develop early-life intervention against maternal stress or other insults.

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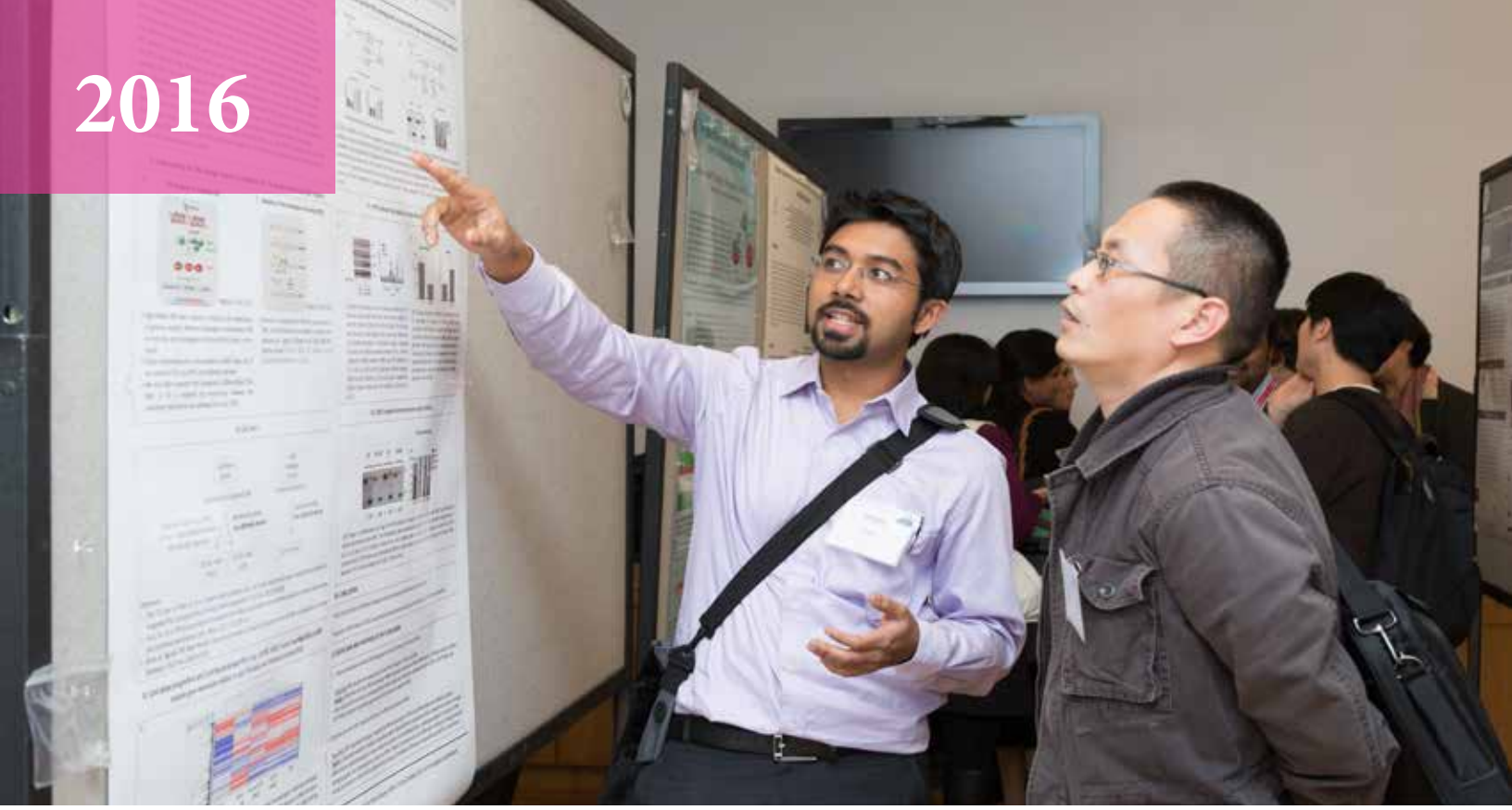

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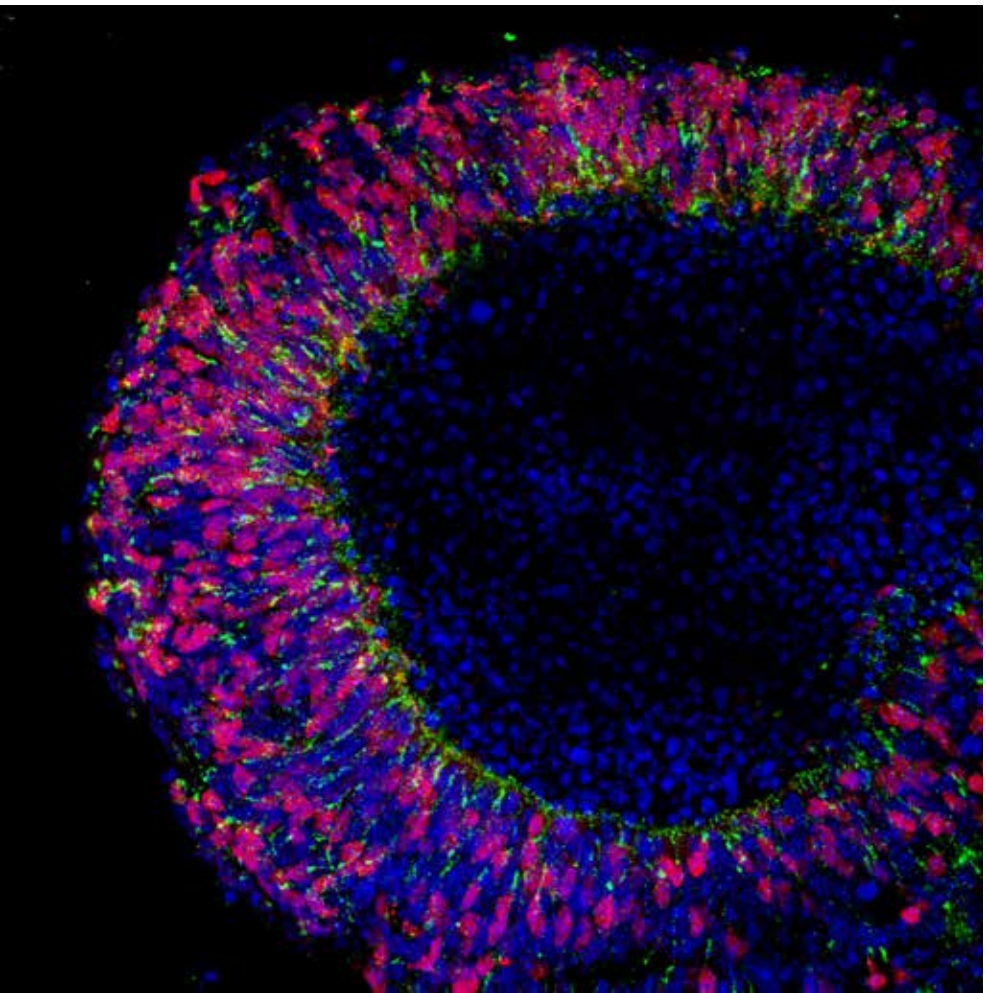

Amnon Bar-Shir, Ph.D.
Johns Hopkins University
2012 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Multiple

Mri-Based Reporter Genes For Non-Invasive Assessment of the Fate of Stem Cell-Seeded Scaffolds

Monitoring the fate of transplanted stem sells is a major challenge in translating stem cell therapy to clinical practice. Although the capabilities of stem cells to self-renew, and to differentiate into many downstream phenotypes has been extensively studied, and their potential to be used for the treatment of a variety of diseases as diverse as neurological conditions or cancer have been demonstrated, their clinical translatability requires a robust imaging methodology to monitor their long-term survival and appropriate function.The research performed under the support of the Maryland Stem Cell Research Fund has led to the development of a novel approach to the non-invasive imaging of reporter gene expression using MRI, with the purpose of monitoring the fate of transplanted therapeutic stem cells. We describe a novel genetically encoded reporter system for both opticalimaging and MRI, which can be used for non-invasive monitoring of transplanted stem cells seeded in Hyaluronic Acid (HA)-based scaffolds. In vivo monitoring of on HA hydrogel using MRI: In the first stage of this project (Year 1), we have developed and demonstrated a new approach, using magnetic resonance imaging (MRI), to the noninvasive in vivo monitoring of dynamic changes in a Hyaluronic-Acid (HA) hydrogel scaffold, which is a supremely supportive material for transplanted stem cells. The capabilities to monitor hydrogel scaffold non-invasively with MRI, after its implantation, provides important information about the dynamic changes over time in vivo, which may contribute to the optimization of hydrogel and transplantation strategies for stem cell therapy. Genetically encoded MRI reporter system: The fluorescent nucleoside pyrrolo-2'-deoxycytidine (pyrrolo-dC) was used as a reporter probe (imaging agent) for both optical imaging and MRI, while the Drosophila melonogoster 2'-deoxynucleoside kinase (Dm-dNK) gene was used as the reporter gene. Pyrrolo-dC (the reporter probe), as is true of other non-natural nucleosides, crosses the cell membrane by facilitating cellular nucleoside transporters. While phosphorylated by the Dm-dNK (reporter gene), the resultant pyrrolo-dC monophosphate accumulates in the cells, as its negative charge prevents its cellular export. Thus, the accumulation of fluorophore in the cytoplasm allows the sorting of cells expressing the Dm-dNK, using a fluorescent-activated-cell-sorting (FACS) based methodology. The main advantage of using FACS for the suggested approach is that the population of the transplanted cells can be enriched with cells expressing the reporter gene. Interestingly, we found that pyrrolo-dC can be used as an MRI reporter probe since it generates strong signal using Chemical Exchange Saturation Transfer (CEST) contrast mechanism.We assessed the imaging properties of pyrrolo-dC (fluorescent and MRI), its accumulation in cells expressing the Dm-dNK, and the capability to sort cells expressing Dm-dNK by flow cytometry (FACS). The ability to monitor the Dm-dNK reporter gene expression in vivo using both optical imaging (using two-photon fluorescent microscopy) and MRI were demonstrated. Mouse glial-restricted precursor (GRP) cells were used to demonstrate the stable expression of the Dm-dNK reporter gene in stem cells. We are now at the stage of combining both MR|-based approaches that have capabilities to monitor dynamic changes in an HA hydrogel, and imaging reporter gene expression in transplanted cells. By accomplishing this, we hope to establish a robust, MR|-based, noninvasive, real-time imaging methodology for the in vivo monitoring of stem cell-seeded scaffolds. The tools developed in this study will help to optimize the composition of the embedding hydrogels for maximal support of the implanted stem cells. Overallduring the time of the MSCRF postdoctoralfellowship we have published four papers (PI: Amnon Bar-Shir as first author) and several others are in preparation and will be submitted soon.

Jeff Bulte, Ph.D.
Johns Hopkins University
2012 Investigator Initiated Award Budget: $683,864
Disease Target: Cancer

Co-Encapsulation of Human Mesenchymal Stem Cells and Islet Cells for Treatment of Type 1 Diabetes

Microencapsulation of íslets has been a widely applied technique for immunoprotection of transplanted islets,bobviating the need of using toxic immunosuppressants. However, normoglycemia in non-human primates and humans can Senerally only be maintained for limited periods. Whe wanted to compare our previously performed studies on díabetic mice, where we could successfully suppress the host immune response by co-encapsulation of human mesenchymal stem cells, to similar studies in (diabetic) pigs. To this end, we performed a systemic swine study on the host immune response towards alginate magnetocapsules containing magnetic resonance imaging (MRl)-visible iron oxide nanoparticles and human islet cells co-encapsulated with human mesenchymal stem cells (MSCs). Fluoroscopic, ultrasound and MRI were used for image-guided delivery in 5 different transplantation sites: the portal vein, liver capsule, kidney capsule, skeletal muscle and subcutaneous skin. Microcapsules cross-linked with clinically approved protamine sulfate and coated with heparin significantly reduced blood clotting for 4g hours. A robust host response was observed for all transplantation sites. Co-encapsulation of hMSCs did not result in an attenuation of the overall immune response, possibly by increasing the load of xenografted cell mass. We concluded that there are major differences between species in terms of graft rejection, We then decided to go back to the drawing board and make improved alginate capsules that are less immunogenic, using chemical purification techníques. After stimulation of Thpl cell lines, we observed that the chemically purified alginate did not evoke an immune reaction, while non-purified alginates showed a MyD88-independent activation of approxim atelv zoyo, When the Thpl-XBlue-MD2-CD14 cell line was stimulated, IGAP induced a lessened immune reaction of 3% when compared with the negative controls. We furthermore tested three commonly used cross-línking cations, i.e., calcium (Ca2+), barium (Ba2+), and strontium (Sr2+). We obserued an immune activation, which was MyDggdependent, when barium or calcium where used in combination with IGAP; however, this activation was not significant when strontium was used, either with LVGP or with IGAP. No significant NF-xB activation that was non-MyD88-dependent was observed with any alginate or cation. The use of different ions did not change significan¡y the strength of alginate beads in LVGP or IGAP alginates (Fígure 2c). tGAp, with a high molecular weight, and LVGp, with a low molecular weight, were then selected to produce capsules due to their rãsistance to mechanical deformation. Calcium and strontium were selected as binding ions because of their low immune activation. We plan to obtain further funding to repeat our mesenchymal stem cell/islet co-encapsulation studies in (diabetic) swine.

Christopher Donnelly, Ph.D.
Johns Hopkins University
Mentor: Jeffrey Rothstein, Ph.D.
2012 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: ALS

Development of an Antisense Oligonucleotide Therapeutic Utilizing Stem Cell Derived Patient Astrocytes to Treat ALS and Dementias Caused by C9ORF72 Expanded Hexanucleotide Repeat

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig‘s disease in the United States, is the most common fatal motor neuron disease and the third most common neurodegenerative disease in the Western World. Progressive muscle wasting and paraiysis characterize ALS. The disease is fatal with no effective therapy and the median survival time for patients is 3-5 years with death typically due to respiratory failure. Only 10% of ALS patients exhibit some familial history of the disease, while the remaining 90% develop the disease sporadically. There is no single disease-causing mutation for ALS, rether, genetic analysis of familial ALS cohorts have identified approximately two dozen mutations in various gene families associated with the disease. Until recently, these mutations cumulatlvely accounted for only 25% of all familial ALS and an extremely small portion of sporadic ALS cases. However, the field drastically changed ln 2011 when two independent labs discovered a hexanucleotide repeat expansion in the first intron of the C9ORF72 gene. This GGGGCC repeat expansion has since been identified as the most common known genetic cause of ALS accounting for up to 50% of familial and 20% of sporadic ALS. ln additlon, the C9ORF72 mutation represents the most common genetic abnormality in familial frontotemporal dementia (30%), and has also been associated with olher neurodegenerative disorders, including Parkinson‘s disease, Huntington‘s disease-like syndrome as well as Alzheìmer‘s disease. Taken together, the C9ORF72 repeat expanslon is the most widespread neurodegenerative disease mutation identified to date. This work aimed to understand the disease-causing mechanisms underlying the C9ORF72 mutation. To achieve this, we generated induced pluripotent stem cells (iPS) from C9ORF72 ALS patient fibroblasts and differentiated them into motor neurons, Using this model, we were able to characterize the affect the repeat expansion mutation in living human neurons. Our data showed that the C9ORF72 mutation reduced the expression of the C9ORF72 gene and generated toxic products in the form of molecules called RNA We thoroughly characterized the C9ORF72 iPSC neurons and compared them to C9ORF72 ALS brain tissue and found a number of genes that were altered in both the |PSC neurons and motor cortex of C9ORF72 ALS patients. We also found that the C9ORF72 IPSC neurons exhlbited increased sensitivity to extracellular stressors, including glutamate. In collaboration with lonis Pharmaceuticals we next attempted to correct the C9ORF72 mutation in |PSC neurons derived from C9ORF72 ALS patients by targeting and silencing the mutation with a molecules called AntþSense Oligonucleotide (ASO) therapeutics. Employing these molecules, we were able to silence the mutated gene and correct the levels of abenantly-expressed genes that were found In both the C9ORF72 iPSC nêurons and ALS patient tissue. Moreover, we found that these ASOs rescued sensitivity to extracellular glutamate stress in the C9ORF72 ALS iPSC motor neurons. In later studies employing these iPSCe motor neurons from C9ORF72 ALS patients, we found that a key cellular process called nucleocytoplasmic transport was dysfunctional and were able to correct this by targeting the mutation.

DaWei Gong, Ph.D.
University of Maryland, Baltimore
2012 Investigator Initiated Award Budget: $600,000
Disease Target: Lipodystrophy

Towards Modeling Pathogenesis and Treatment of Congenital Generalized Lipodystrophy Using Patient-Specific iPSCs

Congenital Senerallzed lipodvstrophy 1 (CGL1) is a monogenic disease due to the deficiency/gene muta¡on of the enzyme 1-Acylglycerol-3-Phosphate O-Acyltransferase 2 (ACPAT2). Patients with CGL1 lack fat tissue and are usually with diabetes and dyslipidemia. So far, there is no effective treatment for the disease, due to, in part, the lack of disease adipocytes for research. lnduced pluripotent stem (iPS) cell technology offers a great promise for patient specific cell-based therapy for many monogenic diseases including CGL1. The main goal of this grant is to establish human CGL1 iPS cells, to differentiate them into mesenchymal stem cells (MSC) and adipocytes and to investigate whether replenishment of the wild-type gene can correct the disease phenotype in vitro and in animal. Main research results are summarized below, Towards the research goals, we first established iPSC celllines using skin cells derlved from CGL1 patients. The CGL1 iPSCs were characterized by expressing typicalembryonlc stem cells markers and by teratoma formation when injected into immunocompromised mice. We, next, establlshed a protoco! to differentiate human iPSCs into MSCs, which ln turn differentlate into adlpocytes. Comparison of CGL1 iPSC-derived MSCs with normal iPSC-derived MSCsshow that the former cells have a significantly decreased adipogenic potential. However, over expresslon of peroxisome proliferator- activated receptor gamma (PPARg), the adipogenlc master gene, in the disease MSCs restored the adipogenic potential. Thus, we found that the main defect ln adipogenesis of CGL1.MSCs is at a step before PPARg inductlon and/or defect in inducing PPARg expression. To prepare for cell-based replacement therapy, we also made iPSCs from mouse embryonic fibroblasts of AGPAT2 (-/-) knockout mice, which mimics CGL1 disease, characterized the iPSCs, and derived MSCs through differentiation.The mouse CGL1 MSGs lack the adipogenlc potent¡alas do the human MSCs. Significantly, virus-mediated AGPAT2 genes in CGL1 MSCs restore their adipogenic potential, indicating that the replenishing CGLl cells with AGPAT2 could be a therapeutic approach for the disease. We then implanted AGPAT2 overexpressing CGL1 MSCs, CGL1 MSCs and normal MSCs into immunocompronrlsed mice subcutaneously. lnjected cells were analyzed three weeks after implantation. Unexpectedly, implanted MSCs from all the groups differentiated poorly into adipocytes, preventing us from determining whether replenishment of AGPAT2 could restore CGL1 adipogenic potential in vivo and correct the disease phenotypes in AGPAT2 {-/-} mice. We continue to explore a better protocol to differentiate iPSC derived MSC into adipocytes ln vivo at present. Nevertheless, in the study we established human and mouse CGL1 iPSCs, discovered a mechanism why AGPAT2 deficiency leads to defect in adipogenesis and demonstrated that defect can be corrected by overexpressing AGPAT2 in vitro. Notably, whlle we are working on the CGL1 projects, we also made several scientific side products.We established NPC1 disease-specific iPSC celline and differentiated them into neutons. During the study of iPSC differentiation, we found that Elabela, a new stem cell hormone, is critical for cardiomyocytes differentiatlon at the stem cells tage and it can improve heart performance in heart failure model in mice. Thus, Elabela is potentially a therapeutic for heart failure.

Pinar Huri, Ph.D.
Johns Hopkins University
Mentor: Warren Grayson, Ph.D.
2012 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Bone Disease

Development of an Antisense Oligonucleotide Therapeutic Utilizing Stem Cell Derived Patient Astrocytes to Treat ALS and Dementias Caused by C9ORF72 Expanded Hexanucleotide Repeat

Approximately 6.2 million fractures occur each year in the United States alone and ca. 15% of them require some kind of bone transplantation. Moreover, it is predicted that the percentage of persons over 50 years of age affected by bone diseases will double by 2020. The therapeutic regeneration of these cases create an economic burden of over $1B annually. The current standard therapy for bone defects remain the autologous grafts obtained from the healthy portion of the patient‘s skeleton. Autografts have obvious limitations such as being limited in availability, and cause additional problems including donor-site morbidity, increased operation times and blood loss. Therefore, more feasible alternative bone graft materials are needed for therapy. Tissue engineering (TE) could be an ideal solution to the worldwide shortage of bone substitutes. TE utilizes autologous cells, biodegradable scaffolds and growth factors to guide cell behavior within the construct to engineer autologous grafts. The current challenges of the strategy include the lack of proper cell sources, as well as the generation of functional vascular supply with compromises graft integration and hinders the potential of engineered bone substitutes for success clinical applications. In this study, the approach is to use human adipose-derived stem cells (ASCs)as a multipotent easily accessible, abundant and clinically-relevant cell source to give both vascular and osteogenic compartments of an engineered vascularized bone graft through the spatio-temporally controlled release of growth factors. We grew human ASCs on 3-D scaffolds prepared from poly (PCL.) which ls a biodegradable, thermoplastic polyester found to be suitable for using long term load bearing applications. PCL was either 3-D printed using a custom-made 3-D printer system recently developed in our laboratory or produced by porogen leaching method to obtain scaffolds with varying internal architecture. Scaffold pore size was controlled by leaching of custom-made paraffin particles with three different size ranges. Scaffolds produced by teaching these particles exhibited highly interconnected pores and rough surface structures that were favorable for cell attachment and ingrowth. The osteogenic response of hASCs was evaluated following up to 3 weeks of culture using biochemical (ALP, Ca2+/DNA content), mechanical (compression test) and histological analysis. It was observed that while the total number of cells was similar for all scaffolds, the cell morphologies and osteogenesis patterns were affected by the scaffold pore size. ASCs were able to bridge smaller pores and grow uniformly within these scaffolds (P200) while they grew as a layer along the periphery of the largest pores (P1000). The cell-biomaterial interactions specific to the latter case gave rise to an enhanced osteogenic response. The ALP activity and Ca2+ deposition were doubled in P1000 scaffolds as compared to P200 scaffolds. A significant difference was observed between the compressive strength of unseeded and seeded P1000 scaffolds. Therefore, we demonstrated that the use of scaffolds with pores that are in the range of 1 pm enhances in vitro osteogenesis, which may improve their performance in engineered bone substitutes. The optimized internal scaffold architecture is now being used to 3-D print scaffolds providing the anatomical-shape that would present patient-specific engineered grafts. Overall, the functionalized graft produced in this study will present an easily translatable, patient-specific, vascularized bone graft. This project also led to new collaborations with the Plastic Surgery Department of the Johns Hopkins University and we are now generating grafts for craniofacial bone regeneration that will be first tested in a mouse model. Moreover, a new project was started to investigate the potential of ASCs to engineer skeletal muscle by studying their differentiation efficiency into myotubes through collaboration with Orthopedic Surgery Department of the Johns Hopkins University. 1 scientific article was published and 3 more are being prepared and 4 conference abstracts were presented based on the results of this study.

Hongkai Ji, Ph.D.
Johns Hopkins University
2012 Exploratory Award Budget: $230,000
Disease Target: Multiple

Global Prediction of Transcription Factor Binding Sites in Lineage Specific Neuronal Differentiation

This project has two goals. The first goal is to develop a computat¡onal approach for predicting transcription factor binding sites (TFBS) based on gene expression data. Our second goal is to apply this computational approach to study neuronal cell-fate determining regulatory networks during human neural progenitor cell (NPC) differentiation Transcription factors (TFs) play important roles in controlling gene expression in stem cell differentiation. In order to comprehensively understand gene regulatory programs in stem cells and disease, it is crucial to map genome-wide binding sites of all transcription factors in a large number of different conditions and developmental time points. The state-of-the-art technologies for mapping TFBS such as ChIP-seq and DNase-seq coupled with DNA motif analysis cannot analyze a large number of different biological contexts simultaneously. This limitation along with various practical constraints such as lack of sufficient amounts of materials, antibodies, resources or expertise have made it extremely difficult to globally analyze the regulatory network involving many TFs and many biological conditions. Our aim L seeks to provide a solution to this problem by developing a computational prediction approach. We achieved this goal by developing a new method BIRD, Blg data Regression for predicting DNase I hypersensitivity. In BIRD, we use large amounts of gene expression and DNase-seq data generated by the Encyclopedia of DNA Element (ENCODE) Project to train prediction models that can predict genome-wide DNase I hypersensitivity based on gene expression data. Unlike ChIP-seq and DNase-seq, gene expression data are more widely available and easier to collect. We have shown that BIRD prediction models trained using ENCODE data can be applied to new gene expression samples to infer active regulatory elements in the genome. Moreover, combined with transcription factor binding motif information, BIRD allows one to predict genome-wide TFBSs of many TFs simultaneously based on gene expression data, which are easily obtainable for many different biological conditions. We have also successfully applied BIRD to 2000+ publicly available exon array samples collected from the Gene Expression Omnibus (GEO) database to compile a regulome database. A manuscript summarizing these results is being submitted for review and publication. Some computational techniques developed in this Aim have also helped a study of Gata6 transcription factor in stem cell differentiation [L]. For our second goal, we have generated time-course exon array data by differentiating induced pluripotent (iPS) cells into dopaminergic neurons. Gene expression exon array data were collected at day 0,I0,20 and 32. Applying BIRD to these data, we predicted genome-wide TFBSs. We found that the predicted TF binding activities at the known SOX2 binding sites decreased as cells differentiated, consistent with the role of SOX2 in stem cell differentiation. This validates our prediction approach. We then used the correlation between predicted TF binding activities and the temporal expression pattern of their putative target genes, as well as DNA motif enrichment, to identify important regulators in this differentiation system. This leads to identification of a series of TFs including NRSF, JUN, CREB, NEUROGl, SOXs and POU6F1 among others, that may play important roles in this system. We also predicted the regulatory hierarchy of these TFs. A manuscript describing these findings is in preparation. In summary, this project has developed a new computational approach to predict global gene regulatory network We applied this approach to study the iPSC-NPC differentiation. Our computational method is complementary to existing experimental technologies for decoding regulome which cannot easily scale up to map binding sites of many TFs and many biological conditions. Therefore, in the long run this new approach will help people to better study stem cells and other biological systems.

Gabsang Lee, Ph.D.
Johns Hopkins University
2012 Investigator Award Budget: $690,000
Disease Target: CIPA

Derivation of Functional Nociceptive Neurons From hESC and Its Application to Pain-Disorder Human iPSC

The first part of our final report is for development of differentiation protocol for peripheral sensory neurons. Previous protocol can take more than 6-7 weeks and must use mouse feeder layers. Our new cost-effective, xenofree and relatively fast protocol, we use chemical compounds (BMP/TGF inhibitor, Wnt activator, Notch inhibitor and FGF inhibitor) to produce sensory neuron precursors within 2 weeks of differentiation. More importantly, we can FACS-purify these sensory neuronal precursors with TrkA antibody. With this new methodology can be applicable to hiPSC. These sensory precursors can give rise to CGRP (Calretrin gene related protein) and Peripherin expressing neurons. However, we found that resulting nociceptive neurons are functionally hetergenous, in terms of their responses (different latency and level of amplitudes) to different chemicals (e.g. capsacin, mustard oil, etc.), which could hamper our detailed characterizatlon and application into pain-disorder hiPSCs. Therefore we are currently developing two genetic reporter system in hESCs, such as TRPVl::GFP and MRGPRXL::GFP, using brand-new genome editing technology (CRISPR/Cas9). My lab has been working with the CRISPR/Cas9 system for more than L2 months and successfully targeted more than 10 different loci in hESCs and hiPSCs lines. We have cloned homology arms by PCR with genomic DNA of hESCs (H9) and one control hiPSCs (GM01582-L) and finished construction of donor vectors for both genes. Along with multiple gRNA vectors and the donor vector, we nucleofected into the hESCs (H9) and control hiPSCs (GM01582-1) and started to select targeted colonies with Puromycin. Currently we have over 40 different clones for each reporter system, TRPV1::GFP and MRGPRXl::GFP, and are performing genotyping as well as FACS analysis upon differentiation. Identified clones will be used for pain disorder hiPSCs for further characterization and testing their in vivo behaviors. Moreover, the in vivo activities of such TRPVl::GFP+ and MRGPRXl::GFP+ neurons are currently under investigation.

In our preliminary data of our original proposal, we showed the early stage of iPSC colony derived from CIPA fibroblasts. This reprogramming was performed by lentiviral transduction. Afterward, we did have CIPA-iPSC colonies (more than 6 clones from 4 independent CIPA fibroblasts). CIPA disease is caused by mutations in TRKA gene that is responsible for specification and survival of nociceptive neurons. lnitially we thought that this mutation can bias our differentiation system in a dish, but based on neuronal morphologies, marker protein expression (TRKA, BRN3a), we not have CIPA-specific sensory neurons. The transcriptional and translational phenotypes are now emerged and in particular, several known phosphorylation proteins are properly phosphorylated upon NGF stimulation. Very recently we found a way to activate TRKA by using optical stimulation, not with NGF protein. Currently we employ this optical stimulation approach to reverse such CIPA-specific phenotypes. ln addition, we are currently preparing other pain-disease hiPSCs with SCN9A mutations. The fibroblasts of the patients (SNCgA mutation) were acquired from collaboration in Germany where IRB steps have been thoroughly reviewed and approved. The fibroblasts are currently under reprogramming. The advantage of this hiPSC line should be the intact differentiation of nociceptive neurons in our system, but should have pronounced deficiency in paininducing chemical detection, due to the functional defect of SCN9A sodium channel. Before we start differentiation of thess new hiPSC clones transgene, we will check any remained Sendai virus transgene as well as the neural propensities of each clone to have similar level as the one of control hiPSCs. ln conclusion, with generous support from MSCRF/TEDCO, my lab has established a very important ground to study human pain disorders. We are very grateful for the support and expect to have a fruitful outcome in coming years.

Guo-Li Ming, Ph.D.
Johns Hopkins University
2012 Exploratory Award Budget: $690,000
Disease Target: Schizophrenia

Toward Correction of Neurodevelopmental Defects Of Neurons Derived from Patients with Mental Disorders

Many neuropsychiatric disorders, including schizoaffective disorder, schizophrenia, autism spectrum disorder and major depressive disorders, are comprised of a diverse array of symptoms and have a complex but poorly understood etiology. Both genetic and environmentalfactors play a role in disease onset and progression. Although there is no single genetic basis underlying these disorders, more than a dozen of different genes have been identified in recent years that appear to increase vulnerability for developing these disorders. Among these, DISC1 (disrupted-in-schizophrenia 1) has emerged as a prominent risk gene for schizophrenia and a spectrum of affective disorders. Originally identified in a large Scottish family, mutations in DISC1 have since been associated with mental illness in several families around the world, including an American Family. The advent of cellular reprogramming in which pluripotent stem cells (iPSCs) are generated from adult skin biopsy samples obtained from living patients, has created an exciting opportunity to investigate the origin of many complex neuropsychiatric diseases, as patient brain cells are not accessible for research. Taking advantage of this technology, we have generated iPSCs from multiple members of the American family in which a specific mutation in DISC1 is associated with schizophrenia, schizoaffective disorder, and major depression. Of the seven family members, five individuals harbor the DISC1 mutation and have psychotic symptoms while the remaining two are healthy individuals without the mutation. During 2O12-2O15 funding year, we have made tremendous progress in our proposed research, which resulted in several publications. My lab was the first to report |PSC lines from schizophrenia patients in the field (Chiang et al. Mol. Psychiatry 2011). We have recently identified a number novel phenotypes using patient derived iPSCs harboring genetic risks for major mental disorders, including DISC1 (Wen et al. Nature 2O14) and 15q11.2 (Yoon et al. Cell Stem Cell 2014). We have also initiated high-throughput screens using these iPSC models and have generated humanized mouse models in parallel for system and behavior levels of analysis and validation of drug hits.

Translational potential: Our detailed analysis of human neurons from patients with psychotic disorders and with defined genetic contributions may reveal novel function of disease susceptibility genes and provide insight into their pathological contribution. We envision that our non-biased, high content small molecule screen using human neurons may lead to the development of novel therapeutic strategies.

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

In Vitro Differentiation of Human Induced Pluripotent Stem Cells into B-Cells for Modeling Human Diseases

The goal of this project is to explore a new effective method to differentiate human induced pluripotent stem cells (hiPSCs) into the B-lymphocyte lineage in vitro and to apply this in vitro B-cell differentiat¡on process to model B cell-related human diseases. To pursue these goals, we proposed the following two aims:

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

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

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

Liu, C., X. Bai, J. Wu, S. Sharma, A. Upadhyaya, C. I. M. Dahlberg, L. S. Westerberg, S. B. Snapper,X.Zhao, and W. Song#. 2013. N-WASP is essential for the negative regulation of B cell receptor signaling. PLOS Biol. 11(11):et0OI704.

Seeley-Fallen, M. K., L. J. Liu, M. R. Shapiro, O. O. Onabajo, T-H Tan, A. Upadhyaya, and W. Song#. 2014. Actin binding protein-L links B-cellreceptorto negative signaling pathways. Proc. Natl. Acad. Sci. USA. 111:9881-9886.

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

Steven Zhan, Ph.D.
University of Maryland, Baltimore
2012 Exploratory Award Budget: $230,000
Disease Target: Multiple

Modulation of Homing and Engraftment of Hematopoietic Stem Cells by I-BAR Proteins

The Specific Aims:

Characterize the role of MIM in human HSPC homing, engraftment and mobilization Development of small molecules targeting MIM dimerization

Summary of the Progress:

Verification of the role of MIM in the trafficking of HSPC: We have investigated the trafficking behavior of HSPCs with a disrupted MIM gene and observed that cells ability of cells derived from MIM knockout mice to home to the lymphoid organs and to mobilize from bone marrow (BM) into the blood. This investigation has revealed that MIM-/- hematopoietic stem and progenitor cells (HSPCs) were able to home the BM of lethally irradiated WT mice at an efficiency 150% of that of wild type donor cells. In addition, leukocytes, including monocytes and lymphocytes derived from the BM of MIM-/- mice also exhibited a higher homing activity to BM or spleen than did those of wild type cells. In an effort to understand the mechanism for the increased homing activity, we examined the response of MIM-/- cells to SDF-1, the primary chemokine responsible for the interaction of HSPCs with bone marro\ry niches. We observed that MIM-/- cells exhibited a greater mobility toward SDF-1 and stronger adhesion to fibronectin in vitro. Consistent with this increased motility and adhesion, MIM-/- cells, these cells expressed a higher level of CXCR4, the receptor for SDF-1. Furthermore, MIM-/- cells showed increased signaling of CXCR4 within MIM-/- cells, including activation of small GTPases Rac and Cdc42 and phosphorylation of p38 MAP kinase. We also examined the response of MIM-/- cells to CXCR4 antagonist AMD3100, and found that MIM-/- cells become hypersensitive to AMD3100. Upon treatment with AMD3100, MIM-/- mice contained mobilized HSPCs 5 times that of wild type mice. Hence, our data demonstrate that MIM plays an important role in the modulation of trafficking of HSPCs and implies that MIM is a suitable target at which one may increase the collection of HSPCs for clinical applications.

Development of small compounds targeting MIM: To modulate the function of MIM within cells, we have designed several short peptide derivatives to target I-BAR dimerization. One of these derivatives had a cyclic configuration with a potency to disrupt the dimerization of MIM or ABBA proteins in vitro, and to be readily internalized into cells. Exposure of cells expressing MIM-I-BAR to this compound abolished increased susceptibility to paclitaxel and partially inhibited the I-BAR-mediated endocytosis. Our data suggests that this cyclic peptide can be used as a tool to study the function of intracellular MIM and as a lead to develop a therapy targeting human diseases involving abnormal MIM expressions.

Peter Andersen, Ph.D.
Johns Hopkins University
Mentor: Chulan Kwon, Ph.D.
2013 Post-Doctoral Fellowship Award Amount: $110,000
Disease Target: Congenital Heart Defect, Myocardial Infarction

Identification of Chamber-Specific Cardiac Progenitor Populations

This research has been completed as planned. The findings have been presented in three different manuscripts. In two of these, I describe how undifferentiated cardiac progenitor renew in the 2nd pharyngeyal arch before the migrate into the developing heart and differentiate into cardiac cells, thus providing a stem cell-niche paradigm for the first time in cardiovascular biology (elife 2014. PMID: 26274057 and PMID: 24843018). The third manuscript describes how deficiency of a protein called Alstrom Protein 1 is the primary cause of mitogenic cardiomyopathy (Nature Communications, 2014, PMID: 24595103). In addition, I obtained a patent based on these findings (#US20140179763 A1). Finally, I have a manuscript submitted to the scientific journal Nature Biotechnology describing how cardiac progenitors can be identified and isolated from pluripotent stem cell cultures. During the project period, I have presented my findings at several national and international meetings.

Jing Cai, Ph.D.
Johns Hopkins University
Mentor: Duojia Pan, Ph.D.
2013 Post Doctoral Fellowship Award Budget: $110,000
Disease Target: Colorectal Cancer

Hippo Signaling In Intestinal Stem Cell Homeostasis and Carcinogenesis

Itestinal stem cells (ISCs) maintain the intestinal homeostasis, perturbation of which may lead to pathological conditions, such as ulcerative colitis and colorectal cancers. While my recent studies have unequivocally implicated the Hippo signaling pathway as a critical mediator of intestinal regeneration and tumorigenesis, the specific cell type upon which Hippo signaling exerts its function in regulating intestinal homeostasis remains to be defined. Inactivation of adenomatous polyposis coli (APC), mutations of which cause familial adenomatous polyposis (FAP), leads to expansion of ISCs and colorectal cancers. Researchers ¡n the FAP field generally believe that APC functions by targeting p-catenin for degradation and that hyperactivation of β-catenin accounts for tumorigenesis after APC is mutated in FAP patients. Thus, major efforts have focused on β-catenin as a therapeutic target for FAP. However, this approach has so far been unsuccessful. Therefore, there is an urgent need for the discovery of novel therapeutic targets against colorectal cancers. My proposed studies have been designed to address these critical questions.

In my fellowship application, I proposed 3 specific aims as follow:

Aim 1: To investigate the role of the Hippo signaling pathway in intestinal stem cell (ISC) self-renewal.
Aim 2: To study the role of Adenomatous Polyposis Coli (APC) in regulating YAP protein level in human colon cells.
Aim 3: To examine the role of Hippo pathway in human colon cancer stem cells.

For Specific **Aim 1**, I have analyzed ISC markers (Lgr5 and Bmi1) in Sav1-deficient small intestine compared to wildtype by real-time PCR but didn‘t observe any change in transcription levels of these marker genes. I have also examined Lgr5-positive ISCs in Sav1-deficient mice as an alternative approach, since Lgr5-eGFP-CreERT2 represents a more sensitive reporter. However, no significant change in the number of ISCs was observed either, suggesting that activation of YAP does not promote ISC self-renewal under physiological conditions. I will further investigate whether Hippo signaling plays an essential role under pathological conditions, such as intestinal tumorigenesis and regeneration.

For specific **Aim 2**, I have found that APC plays an essential role in suppressing the YAP oncogene through the Hippo signaling pathway, Mechanistically, APC functions as a scaffold protein to facilitate the interaction between Sav1 and Lats1. Most importantly, I found that YAP is absolutely required for adenoma development upon inactivation of APC using mouse genetics, suggesting that YAP may represent a novel drug target in FAP. These finding has been published in Genes & Development,2015,

For specific **Aim 3**, I proposed to studies the role of Hippo signaling in isolated cancer stem cells from human colorectal cancers by introducing YAP siRNA or a dominant negative form of TEAD2 in lentiviral vectors into these cells. State-of-the-art tools have since been developed to facilitate my studies, including intestinal organoids derived from human pluripotent stem cells (2014) and Crispr/Cas9 genome editing technology (2013). Therefore, as an alternative approach to my proposed experiments, I am currently using Crispr/Cas9 to knockout Sav1 and YAP in human pluripotent stem cells and developing the intestinal organoid culture to study the role of the Hippo signaling pathway in human ISCs.

Translational potential:This project bears direct relevance to understanding the pathogenesis and treatment of colorectal cancers, Since deleting β-catenin leads to intestinal cell death, while deleting YAP causes no symptom under normal conditions, YAP might be a better choice as a therapeutic target for FAP. In the near future, I will perform a drug screen to search for potential chemicals that can restrict human colon cancer stem cell growth in a YAP-dependent manner.

Hugo Guerrero-Cazares, Ph.D.
Johns Hopkins University
2013 Exploratory Award Budget: $230,000
Disease Target: Multiple

Controlling Migration of Human-Derived Fetal Neural Stem Cells Via Slit Proteins in a Demyelination Animal Model

During our project we were able to **(1)** Determine the expression of Robo1 and Robo2 in the subventricular zone neurogenic niche of human fetal brain; **(2)** Establish primary cultures of human neural progenitor cells and determine the expression of Robo receptors by these cells; **(3)** Determine the response of human neural progenitor cells to Slit2 stimulation. Slit2 exerts a chemorepellent response in human neural progenitor cells migration; **(4)** Robo1 expression is necessary for the cell migratory response to Slit2; **(5)** The GTPases Rac1 and CDC42 show a decrease in their active form presence upon slit2 stimulation of human neural progenitor cells; **(6)** In vivo, human neural progenitor cells, implanted into the SVZ of rodents, are able to reach the olfactory bulb 10 days after implantation. The ability of these cells to reach the olfactory bulb is impaired when Robo1 expression is knocked down using siRNA vectors.

Human neural progenitor cell (NPC) migration within the subventricular zone (SVZ) of the lateral ganglionic eminence is an active process throughout development. The migration of human NPCs from the SVZ to the olfactory bulb during the fetal stages resembles what occurs in adult rodents. As the human brain develops during infancy, this migratory stream is drastically reduced in number and becomes barely noticeable in adults. The mechanisms regulating human NPC migration are unknown. The Slit-Robo signaling pathway has been best defined as a chemorepulsive cue involved in axon guidance and neuroblast migration in rodents.

Slit and Robo proteins expressed in the rodent brain help guide neuroblast migration from the subventricular zone (SVZ) through the rostral migratory stream to the olfactory bulb. Here, we investigate the role that Slit and Robo proteins play in human-derived fetal neural progenitor cells (hfNPC). We describe here, for the first time, that Robo1 and Robo2 isoforms are expressed in the human fetal SVZ. Furthermore, we demonstrate that Slit2 is able to induce a chemorepulsive effect on the migration of hfNPCs derived from the human fetal SVZ. In addition, when Robo1 expression is inhibited, hfNPCs are unable to migrate to the olfactory bulb of mice when injected in the anterior SVZ. Our findings indicate that the migration of human NPCs from the SVZ is partially regulated by the Slit-Robo axis. This pathway could be regulated to direct the migration of NPCs in human endogenous neural cell therapy.

Xiaofeng Jia, Ph.D.
Johns Hopkins University
Mentor: Duojia Pan, Ph.D.
2013 Exploratory Award Budget: $230,000
Disease Target: Peripheral Nerve Injury and Regeneration

Electrical Stimulation on Neural Crest Stem Cell Transplantation in Nerve Regeneration

About 360,000 people in the USA alone yearly suffer from damage to peripheral nerves. Peripheral nerves are essential to movement and sensation. Injuries to peripheral nerves are often treated surgically. If the damage is severe, the nerve with a large affected injured gap can only be repaired by transplanting a portion of a less important nerve, usually from the leg, chest, or abdomen. This serves as a bridge connecting the two ends of the severed nerve. This technique is still considered the "Gold standard" because it has the best treatment outcome to date. But it has limitations because donor sites are often limited and problems can occur at the donated site. Even with the grafts, functional recovery is often not perfect because nerves do no regenerate well or quickly enough to regain satisfactory function from a large affected injured gap. We have shown stem cell based approaches promote nerve regeneration after nerve injury. However, how electrical stimulation on transplanted stem cells works remains largely unknown. The research aims to develop and improve a cell-based therapeutic approach with electrical stimulation to improve nerve regeneration, and therefore, addresses one of the most challenging aspects for repair of traumatic nerve injuries. The research will improve the understanding of how stem cells function in nerve regeneration. It will also address a necessary step in before moving towards clinical trials combining stem cell therapy and electrical stimulation. The research shown that electrical stimulation together with stem cell transplantation significantly improved nerve regeneration after injury. Outcomes included increased muscle recovery, better histomorphologic results, and behavioral function recovery, which were better not only than either stem cell transplantation or electric stimulation alone, also comparable to the autograft repair group. Combined stem cell transplantation plus electrical stimulation promoted the nerve regeneration after nerve repair It represents an effective and safe approach for better outcome after peripheral nerve repairs. The project will advance the knowledge of stem cell biology and accelerate the use of stem cell therapies for patient use, and offer better functional outcomes. It will also help in developing optimal treatment protocols for orthopedic surgeons specializing in nerve repair.

Sunjay Kaushal, Ph.D.
University of Maryland, Baltimore
2013 Investigator Initiated Award Budget: $690,000
Disease Target: Heart Failure

Characterization of Resident Cardiac Stem Cells in Neonates

My laboratory focuses on a small population of undifferentiated cells with the characteristics of stem cells (CSCs) present throughout the human heart. Recently, our novel data in a nude rat myocardial infarction (MI) model demonstrated that human cardiosphere derived cells (hCDCs) isolated from neonatal hearts produced greater recovery of ventricular function than did hCDCs isolated from adult donor hearts. Since differentiation of transplanted hCDCs into mature cardiac lineage cells occurs only at a low frequency in this model, cytokine release by the hCDCs may play a dominant role in the functional recovery of the infarcted myocardium. Specifically, our data suggested higher VEGF and ANG-2 secretion from neonatal-derived hCDCs as compared to adult-derived hCDCs stimulates functional preservation/formation of neovascularization. Elucidating the functional mechanisms of hCDCs is the purpose of this grant.

Specific Aim 1. To determine if neonatal-derived hCDCs stimulate angiogenic cytokines in vitro and in vivo. (years 1-2). Hypothesis: Neonatal-derived hCDCs potently stimulate myocardial recovery, principally via increasing neovascularization. We have recent evidence that the regenerative effects may occur soon after the hCDCs were injected in the infarcted rodent myocardium. Because this early recovery cannot be explained by the occurrence of meaningful regeneration resulting from cardiomyogenic differentiation, we postulated that it was achieved through protection /recovery of the ischemic myocardium by paracrine mediator(s) released in situ by the hCDCs. To test our hypothesis, we first assessed the effects of conditioned media from cultured hCDCs in vitro on HUVEC cells to form endothelial cells, an assay for neoangiogenesis. Exposure to neonatal-derived hCDCs-conditioned medium did significantly increase the endothelial tube formation by the HUVEC cells in comparison to adult-derived hCDCs conditioned media. We next demonstrated that a single dose of the condition media derived from neonatal hCDCs significantly recovered the left ventricular ejection fraction in the rodent myocardial infarction model in comparison to control and condition media from adult-derived hCDCs. The histological analysis demonstrated that the condition media derived from neonatal hCDCs demonstrated decreased myocardialscar, increased myocardial viability, and increased neovascularization in the ischemic left ventricle. These results confirmed that the secretome is the main functional unit of the hCDCs that mediates myocardial functional recovery.

Specific Aim 2. To determine the contribution of bone marrow-derived EPCs to neonatal-derived hCDC-mediated neovascularization (Years 2-3). Hypothesis: The observed neovascularization is mediated by EpCs that are mobilized to the myocardium for functional recovery. By using a tie2-lacZ mouse, we demonstrated that transplanted neonatal-derived hCSCs into the infarcted lead to recruitment of endotheial progenitor cells (EPCs) from the bone marrow. These EPCs than participated in increasing the number of arterioles and neovessels in the infarcted myocardium. We will know determine the molecular signals that are responsible for this EPC recruitment.

Specific Aim 3. To determine the role of HIFlq in directing neonatal-derived hCDC neovascularization (years 2-3). Hypothesis: Upregulated in neonatal-derived hCDCs, HIFlc signaling regulates expression of angiogenic cytokines in these cells. We have found that neonatal hCDCs express HIFlc at a significantly higher level under hypoxic condition compared to adult cells. Additionally, our hypoxia induced conditioned media from hCDCs secreted more VEGFA and gave rise to longer and more robust endothelial formation in comparison to the conditioned media exposed to normoxia. Both suggested that the potential role of hypoxia in mediating hCDC neovascularization. Overexpressed HIF1α in adult derived hCDCs has demonstrated that these modified CDCs when transplanted in the myocardial infarcted myocardium generated more myocardial recovery through increased neovascularization in comparison to nonmodified adult derived hCDCs. Knock-down of HIF1α, in neonatal derived hCDCs has demonstrated that these modified neonatal hcDCs had decreased myocardial recovery and decreased neovascularization of the myocardium in comparison to, non-modified transplanted neonatal hCDCs. Taken together, these results showed that HIF1α, plays a critical role in cytokine secretion and thus functional activity of hcDCs.

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

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

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

The approaches were: **1)** Develop and optimize polymeric nanoparticles consisting of 19F-active molecules (19F-Nano) that could permeate human mesenchymal stem cells (hMSCs) without inducing toxicity or affecting MSC bioactivity. **2)** Draft a 19F hMSC-labeling protocol for in vitro and in vivo imaging applications and analyze the in vivo detection threshold of 19F-Nano-labeled hMSCs. **3)** Image and quantify transplanted hMSCs by 19F/1H MRI during cardiac repair.

During the first year of the MSCRF program, the PI's group developed a library of over ten 19F-Nano for in vitro and in vivo preclinical imaging studies (Aim 1) and drafted an hMSC 19F-Nano-labeling procedure that could be completed within 4 hours for in vivo 19F/1H dual MR imaging (Aim 2). Optimized nanoformulations were chosen based on their safety and uptake into MSCs.

In the second year of the MSCRF program, the detection thresholds of 19F-Nano and 19F-Nano-labeled hMSCs were examined (Aim 2), optimized labeling formulations were investigated for their effect on hMSC biological function, including stem cell differentiation (Aim 2), and the in vivo potential of 19F-Nano-labeled hMSC tracking was confirmed in mice (Aim 3). Based on our extensive MR imaging studies in Year 2, we found that the detection signal of 19F-Nano-labeled hMSCs was too low for in vivo cellular tracking. This is an unfortunate drawback of 19F imaging, as we identified in the „Pitfalls" section of our research proposal. Therefore, we developed an alternative approach to label hMSCs with 19F-Nan via biorthogonal copper-free click chemistry to meet the detection threshold for in vivo cellular tracking. Targetable chemical receptors were artificially induced on the surface of hMSCs and complementary targeting groups were added to the 19F-Nano. Using molecular techniques, we confirmed the stability, safety, and binding efficacy of these artificial chemical receptors on hMSCs. Next, we re-optimized the 19F-Nano formulations labeled with a specific binding group and confirmed a strong 19F MR signal that was proportional to the 19F concentration. 19F-Nano were labeled with fluorescent dyes to easily observe cellular uptake. Using fluorescence microscopy and fluorescence-activated cell sorting analysis (FACS), we verified effective binding between the generated chemical receptors on hMSCs and binding groups on 19F-Nano. Most importantly, we confirmed efficient and fast hMSC-labeling with 19F-Nano using fluorescence and 19F MR imaging. Finally, we explored in vivo tracking of 19F-Nano-labeled hMSCs in mice. We subcutaneously administered 19F (via 19F-Nano)- and dye-labeled cells at a range of concentrations and monitored their fluorescence signals for up to one week after transplantation. We established that about 1,000 hMSCs could be detected in vivo for up to a week. Although we did not monitor cardiac remodeling using this approach, we demonstrate that our approach can achieve an effective in vivo detection limit for transplanted 19F-Nano-labeled hMSCs. Therefore, the project goal was achieved.

Our method can provide extensive new ideas for future stem cell 19F MRI studies. The PI continues to focus on the proposed research, expand research collaborations with established stem cell researchers and clinicians, and compete for research grants to expand stem cell research towards novel stem cell-based imaging and therapy.

Kathryn Wagner, Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
2013 Exploratory Award Budget: $230,000
Disease Target: Muscular Dystrophy

A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation

The overall objective of this completed project was to "develop a 3-dimensional environment for the successful transplantation of stem cells to skeletal muscle." Over the course of the funding period, we completed several major milestones within each of the smaller goals, or "specific aims" associated with this project.

After considering several different potential cell-delivery vehicles such as solid scaffolds and nanofibers, we agreed upon the use of a hydrogel, defined here as a water-based gel composed of both artificial and naturally derived components. An optimal gel would remain liquid to allow for greater injectability with minimal invasiveness, spread evenly across an injected muscle, solidify over time, and eventually degrade. Several different compositions of the hydrogel were tested, each containing different components at various ratios and concentrations. At least half of these tested compositions proved sub-optimal, either killing muscle cells or not allowing for injectability. Ultimately, we agreed upon an optimal hydrogel, which has two components. One component is hyaluronic acid, a branched sugar found in the body, which can form a gel upon exposure to water. Hyaluronic acid, or HA, has been associated with facilitating muscle cell growth, and thus was deemed a natural choice. The other component of the gel is decellularized skeletal muscle extracellular matrix, or dSKM-ECM. This product, which is derived from porcine sources, contains the underlying scaffold proteins, which give structure and support to muscle, while signaling muscle cells to grow. The mixture of these two components allowed muscle cells to grow without killing them, remained injectable and spread evenly across muscle one injected, formed a solid gel over the course of one week, and was completely dissolved by 6 weeks. Most notably, when human muscle cell transplantation was tested in mice, the hydrogel promoted cell survival in the short term, with four times as many cells present after one week when injected with the gel rather than without, and longer-term transplantations showed a greater number of human fibers forming in hydrogel-injected mice.

The collaboration outlined in this project led to the successful isolation and derivation of various human muscle stem cells. Several skeletal muscle stem cell lines were derived from commercially available human embryonic and induced pluripotent stem cells, and characterized for their ability to form mature muscle both in a dish and when transplanted into animals. Entirely new transplantation protocols were developed by our labs, and optimized over a period of several months. We have now demonstrated the capability of these human skeletal muscle stem cells to form human muscle fibers in healthy mice, and they can even replace local mouse stem cells within muscle, forming completely human tissue. However, we have also demonstrated that this regenerative capability is diminished in genetically defective mice, which have muscular dystrophy. The hydrogel previously described above has the potential to reverse this trend, promoting increased transplantation efficiency and human muscle fiber formation but these studies are currently ongoing.

Overall, the advances made by this project demonstrate a potential solution to the low transplantation efficiencies observed in previous skeletal muscle stem cell clinical trials; since each component of the hydrogel is based upon FDA-approved technology, translation of this product is highly feasible. Additionally, as the field of cell therapy continues to mature at the rapid pace observed within the past few years, opportunities for clinical translation of the stem cell derivation strategy derived during this study will also present themselves. The combination of human skeletal muscle stem cells, injectable biomaterial hydrogel, and future work with myostatin inhibitors, is on track to emerge as a novel, highly effective method to deliver human stem cells to skeletal muscle.

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

Cell Replacement for Stroke Sing hiPSC Derived 3D-Organized Cortical Neurons

We focused on cell replacement for stroke using cerebral-specific organized neural assemblies to substitute the ischemia-induced loss of neurons and circuits. Since we are targeting to substitute the stroke injured cortex, the host neuronal cell death was analyzed 1,3, 7, 14 days post occlusion of the distal middle cerebral artery. In host stroke injured cortex, extensive neuronal death was seen 3 day after stroke. The host neuronal death reached and maintained almost the maximal level 7 days post-stroke, at which time point we select for cellular transplantation. To further enhance graft cell survival and neuronal differentiation after transplantation, we have identify a candidate molecule possessed strong effect on promoting differentiation and cell survival based on our neuroprotective gene screening on human neurons preconditioned with OGD (Oxygen-Glucose-Deprivation). Moreover, we have modified and generated more advanced 3D-sandwich organized cortical assemblies and cortical organoid for cell transplantation. The neurons in 3D-organized cortical assemblies and cortical organoid are positive for cortical laminar markers,Tbr1, Ctip2, Brn2, and Satb2 with tendency to segregate into cortical layers.Intracranial injections of hiPSC-derived cortical neural cultures expressing GFP or dsRED 7 days after stroke had been performed including mice (i) transplanted with 3D organized cortical assemblies in matrix;(ii) transplanted with dissociated neuroblasts in matrix; (iii) sham-injected with matrix. Seven days after dMCAO, 3D organized cortical assemblies were transplanted into stroke mice, immunostaining of cryostat sections show transplanted cell in matrix survived and maintained the same pattern as compared to the cortical assemblies in culture with laminar structure and cortical layer markers. Transplanted neurons started to extend their projections to host tissue. We monitored the structure integration of grafted cell with long time post-transplantation. Besides somatosensory tests: adhesive removal test, forelimb placing, corner test and forelimb flexion, motor behavioral assays: pellet retrieval task and cylinder test,we introduced also staircase test and pasta retrieval to assess functional outcome after cellular transplantation. We are now undergoing intensive data analysis and results will be reported in scientific publications.

During the period we conducted the project, we have developed a method for generating cortical neural stem cells and neuronal cells with high quality and billions of cell scalability. We have shared and will continue to share these cell resources in and outside the Lab, which reported in four publicly accessible publications (Cell, 2014,157:472-85; Mol Cell Ther, 2014, 17:2; Cell Rep, 2014, 8:681-8; Stem Cells Transl Med, 2014, 3:888-98) with two manuscripts under submission process and two manuscripts in preparation. Furthermore, we have presented the funded project at various meeting: SFN 2013, MSCRF 2013, 2014, Solomon H. Snyder Department of Neuroscience Meeting 2013, JHU Stem Cell Research Leadership program 2013, JHU Research in Progress Stem Cell / Cell Engineering Meeting, 2014. The availability of generating highly enriched cell populations bring us an opportunity to establish new collaborations with Dr. Hongkai Ji at Johns Hopkins Bloomberg School of Public Health (RNAseq data resources for BIRD ChiP analysis and validation of targets), Dr. Akhilesh Pandey at Institute of Genetic Medicine, JHMI (for proteomic study), Dr. Donald Jeffrey Zack lab at Wilmer Eye Institute, JHMI (for neurotoxicity), Dr. Zhibin Wang at Johns Hopkins Bloomberg School of Public Health (for DNA methylation) and Dr. Mingyao Ying at Kennedy Krieger Institute (for neuron differentiation).

Our established novel neural cell production system can generate cortical neurons with high purity, quality, considerable safety and billions of cell scalability. Transplantation of hES/iPS derived neurons into stroked mouse can improve the functional recovery. This technology can be used in both academics (we have already provided human neurons to more than 4 different laboratories at JHMI for the experimental investigation) and industry for identify compound. Thus, the outcome of this research will potentially lead to expansion of cell-based therapeutic screening, functional drug development and eventually cell transplantation in a range of neurodegenerative diseases in the State of Maryland.

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

Directed Delivery of Therapeutic RNAs into Hematopoietic Stem-Progenitor Cells

The purpose of this project was to develop an SDFI-RBD based Chemo-ARP that will enable researchers and clinicians to specifically deliver therapeutic RNAs into HSPCs and hematopoietic cell lines. Specifically, we designed a clone that fused the protein coding sequence of the SDF1 chemokine with the RNA binding domain (RBD) derived from the hepatitis B virus (HBV). The clone was produced and confirmed by DNA sequence analysis. It was then linearized and transfected into the yeast Pichia pastoris in such a way that it became stably integrated into the P. pastoris genome. This was confirmed by genetic and polymerase chain reaction (PCR) analysis of the P. pastoris chromosomal DNA. Next, we induced production of the recombinant SDF-RBD protein by addition of 1% methanol to cell cultures. This caused production of the protein, which was excreted from the cells into the surrounding medium. The recombinant protein was then purified from the medium using a simple 1 step anion exchange chromatography method. Purified protein was analyzed by using SDS polyacrylamide gel electrophoresis (SDSPAGE) This is shown in Figure 1. The ability of the purified recombinant protein to function as an RNA transfection reagent was assessed by monitoring the ability of this reagent to knock down expression of a target gene in Jurkat cells that express the receptor for SDF1 (the receptor is called O(CR4). Figure 2 shows that performed as well as or better than the commercially available HiPerfect transfection reagent. From that point, we delivered the reagent to the laboratory of our collaborator, Dr. Curt Civin (UMB). They are currently characterizing the ability of this reagent to deliver miR-144 and miR-451 into hematopoietic stem precursor cells to regulate human erythropoiesis by targeting the RAB14 mRNA. The crucial take home from this report is as follows: We successfully developed a strain of P. pastoris that produces the recombinant SDFI-RBD reagent that is capable of delivering small RNAs into cells that express the CXCR4 chemokine receptor. Having established this technology, it is now being characterized for its therapeutic applications.

Stephen Eacker, Ph.D.
Johns Hopkins University
2014 Exploratory Award Amount: $230,000
Disease Target: Autism Spectrum Disorders, Schizophrenia

Translational Targets of mTOR in hNPC Development

Specific Aim 1: What are the targets of IGF/mTOR signaling in hNPCs? We have successfully completed the goals of this Aim. Using acute treatment with Tarin, a potent inhibitor of the mechanistic target of rapamycin (mTOR) protein, we have identified translationally regulated transcripts in culture human neural precursors (hNPCs). We identified numerous, previously identified targets of mTOR including components of the translation initiation machinery and ribosomal proteins. In addition we discovered a number of novel targets that we have verified using an orthogonal technology. To further verify these as true targets, we will conduct further assays using luciferase-based reporter assays. Among the targets discovered as targets of mTOR signaling are the homeobox transcription factors GSX1 and 2. These transcription factors regulate the specification of classes of GABAergic interneurons in mouse and chick, and therefore may be extremely relevant targets for understanding disease where the balance of excitatory and inhibitory neurons are disturbed.

Specific Aim 2: What mTOR target mRNAs promote the conversion of hNPCs to mature neuronal subtypes? GSX1 and 2 represent strong candidates for factors that could be mTOR-dependent regulators of mature neuron subtype diversity. We have decide to pursue a loss-of-function approach to test this hypothesis. Using the CRISPR/Cas9 system we have successfully generated GSX1 and GSX2 single mutants in Hhl ES cell lines. We are currently in process of generating GSX1/2 double mutant lines as the literature suggests that there may be partial redundancy of these two transcription factors. Once these lines are obtained, we will characterize the impact of the absence of GSX1/2 on the diversity of neurons generated during in vitro differentiation into cortical neurons. Our hypothesis is that GSX1/2 mutation should partially phenocopy the mTOR knockdown phenotype described in our proposal.

Translational Potential of the Project: We have developed a novel methodology to generate cortical neurons from hESCs and iPSCs. This methodology comes closer than any previously published method to producing the diverse neuronal populations found within the human cortex. Our results thus far provide a potential mechanism for the observed imbalance of excitation and inhibition observed in schizophrenia and autism. The long-term goal of this work will be identification of compounds to treat neuropsychiatric conditions such as autism and schizophrenia, major classes of disorders for which there are few good treatment options. This proposal will further foster this research which will eventually lead to expansion of cell-based therapeutic screening and drug development in Maryland.

Sonia Franco, Ph.D.
Johns Hopkins University
2014 Exploratory Award Budget: $230,000
Disease Target: Ataxia-Telangiectasia (A-T)

Induced Pluripotent Stem (iPS) Cell-Based approaches for Modeling and Treating Ataxia-Telangiectasia (A-T)

Biallelic mutations in ATM result in the neurodegenerative syndrome Ataxia-Telangiectasia, while ATM haploinsufficiency increases the risk of cancer and other diseases. Current murine and porcine models of A-T do not recapitulate neurodegeneration and other features of A-T, prompting us to develop human iPS cell-based models. However, previous studies had revealed low reprogramming efficiency from A-T and carrier fibroblasts, a barrier to iPS cell-based modeling and regeneration. Here, we tested the feasibility of employing circulating erythroid cells, a compartment no or minimally affected in A-T, for the generation of A -T and carrier iPS cells. To this end, we obtained peripheral blood samples from two A-T families attending the Johns Hopkins Children's Center A-T Clinic (one A-T patient and his carrier parents for each family), after obtaining informed consent. Following a protocol previously developed in the laboratory of our collaborator Dr. Linzhao Cheng, we find that episomal expression of Yamanaka factors plus BCL-xL in blood erythroid cells results in highly efficient iPS cell production in feeder- free, xeno-free conditions for the A-T patients and the carrier parent s. In addition, the iPS cell lines from the first family were characterized in detail and shown to maintain long-term replicative potential, stable karyotypes, re-elongated telomeres and capability to differentiate along the neural lineage in vitro and to form teratomas in vivo. These data has been recently submitted for publication. The iP cell lines generated from the blood o f the second family are frozen and awaiting characterization.

The main translational implications of this work are:
I) it is possible to reprogram A -T and carrier peripheral blood erythroid cells to iPS cells with high efficiency.Therefore this protocol could be widely applied in the future for A-T disease modeling and, in the longer-term, for the development of regenerative therapy.

2) The iPS cells we generated from the patient blood have a normal karyotype and re-elongate telomeres, in contrast to the patients original cells that have chromosomal defects and shorter than normal telomeres (genomic instability). Therefore, reprogramming per se has a beneficial effect because it corrects at least some of the abnormalities that cause tissue dysfunction in somatic A-T cells. These data is important because it suggests that the iPS cells arc probably the best substrates for gene therapy of this disease.

Overall, the work we have done so far provides the reagents and rationale for continued efforts to:

I) Fully characterize the A-T and carrier iPS cell lines that we have generated from the second family, in order to consolidate our findings in a second, independent line;

2) Develop isogenic iPS cell lines by correcting the ATM mutation in the two 1\-T lines, using CRISPR/Cas9-based methods and oligonucleotide templates;

3) Differentiate A -T iPS cells to neural cells and cerebral organoids for disease modeling. In particular, we plan to develop novel protocols to generate Purkinje cells and cerebellar organoids, most affected in A-T. We will then use these models to screen for proteins or compounds that could be used to treat neurodegeneration in A-T. This will be the goal of a future Discovery application to MSCRP; and

4) In the longer-term, differentiate the corrected cells to the neural lineage to test the feasibility of tissue transplantation for regenerative medicine. These studies will establish the requirement for clinical transplantation, such as patient age, cell lineage and number, via of administration and translational end-points for success.

Anna Jablonska, Ph.D.
Johns Hopkins University
Mentor: Piotr Walczak, Ph.D.
2014 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Stroke

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

Studies in rodent stroke models indicate that oligodendrocytes are highly vulnerable to damage after ischemia and their loss likely contributes to secondary neuronal injury. Glial restricted progenitors (GRPs) have been shown to engraft, myelinate and lead to extended lifespan of demyelinated mice following neonatal intracerebro-ventricular (ICV) transplantation. However because stroke requires instant and broad distribution of cells throughout the lesion, we decided that intra-arterial transplantation seems more optimal. Unfortunately, our earlier studies show that naïve GRP cells injected intra-arterially pass the brain vasculature quickly without localization in the brain parenchyma. Previously, we used the Very late Antigen 4 (VLA4)-Vascular Cell Adhesion Molecule 1(VCAM-1) pathway for enhanced homing of GRPs to the brain.

Specific Aim 1 of this study was to transiently overexpress VLA-4 adhesion molecules in primary GRPs using mRNA transfection and assess functionality of the transgene using microfluidic chamber assays. The mRNA-based transfection method is more demanding than use of DNA plasmids, but has a better translational potential due to the lack of DNA integration and genomic alterations. We create a constructs for efficient production of mRNA for integrin α4 and β1 with use of mMessage mMachine ® T7 Ultra Kit (Invitrogen) with additional step of adding ARCA cap to the transcript. This mRNA was used for transfection of primary GRPs. Transgene expression in engineered cells was confirmed at the mRNA (RT-PCR) as well as at the protein level (immunocytochemistry). Engineered cells were compared to GRPs transfected with use of DNA plasminds. We observed that following DNA transfection of both VLA4 subunits, the expression starts at 24h after transfection, and is stable for at least 5 days, where use of mRNA results in protein expression as early as 12h after transfection and decrease after 72h. Maximum expression of VLA4 for engineered cells was 48h for DNA_VLA4+GRPs and 24h for mRNA_VLA4+GRPs.The functionality of the VLA4 transgene was analyzed with the use of a microfluidic channel device coated with VCAM-1protein under sheer stress condition.

We found that both DNA- and mRNA- engineered VLA4+GRPs travel through the channels with a slower speed compared to naïve GRPs, indicating VLA-4/VCAM-1binding. Additionally, average speed of mRNA_VLA4+GRPs was 15% lower in comparison to DNA_VLA4+GRPs, which may be explained by the higher expression level for the mRNA-based transfection method.

Specific Aim 2 was to evaluate applicability of VLA4 overexpression using mRNA for enhanced intraarterial targeting of primary GRPs in MCAO model of stroke. For in vivo assessment of GRP cell homing to the stroke lesion, engineered cells were intraarterially injected in mice 24h after middle cerebral artery occlusion (MCAO). The docking efficiency of luciferin expressing naïve vs. VLA-4 expressing GRPs was tested with bioluminescent imaging. Within the first 5 days we observed reduction of BLI signal in all experimental groups, but the reduction was greatest for naïve GRPs transplanted mice with a complete loss of signal beyond day 5. In animals transplanted with engineered cells BLI signal was detectable until the end of experiment at day 14. Importantly,the BII signal in animals transplanted with mRNA_VLA4+GRPs was almost 1.5-fold higher in comparison to DNA_VLA4+GRPs. At day 14 presence of transplanted cells was confirmed with immunohistochemistry.

Raju Khatri, Ph.D.
University of Maryland, Baltimore
Mentor: Michal Zalzman, Ph.D.
2014 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Osteoporosis, Stem Cell therapy

Increasing the Replicative Lifespan and Quality of Adult Mesenchymal Stem Cells

Human mesenchymal stem cells (MSCs) are multipotent pericytes that reside on blood vessels in all tissues throughout the body. Because of their ability to differentiate into multiple cell types, MSCs have a tremendous potential for applications in the field of regenerative medicine. However, the clinical application of MSCs is still limited by the decline in stem cell quality, differentiation efficiency and replication capacity with physiological aging. This decline hinders reproducibility and feasibility of stem cells treatments. Tonsil tissue has recently been identified as an alternative source of MSCs, but previous studies on tonsil-derived MSCs (T-MSCs) have not been thoroughly investigated to determine their in vitro expansion efficiency and differentiation potential. The main objective of this research focused on generation of novel protocols to isolate and stabilize normal adult stem cells in long-term cultures as a safe, renewable resource for future cell therapies. In this project, we generated and characterized fourteen tonsillar biopsy derived T-MSC cell lines from donors of different medical history, and ages ranging from 3 years to 59 years old. T-MSCs were expanded in culture and characterized by markers of pluripotency (SSEA-4,TRA-1-80 and TRA-1-60) and mesenchymal stem cells (CD44,CD73, and CD90). While a sub-population of isolated T MSCs express pluripotency markers; 99% of TMSCs express the markers of mesenchymal stem cells.Additionally, we defined the differentiation potential of our T-MSCs into bone, cartilage and fat tissue. In the second year of the project we completed a safety study in vivo in NSG mice to exclude cell transformation and tumor formation. Our seven months long study, following a subcutaneous injection, demonstrates that the T-MSCs survive, but do not proliferate or form tumors.

In conclusion, we developed a novel protocol for the isolation of T-MSC from tonsil tissues using minimally invasive harvesting procedures. Our protocols are efficient in isolation of MSCs from adult tissues and yield massive in vitro expansion, which is essential for regenerative medicine.

Sang Hoon Kim, Ph.D.
Johns Hopkins University
Mentor: Hongjun Song, Ph.D.
2014 Post-Doctoral Fellowship Award Budget: $110,000
Disease Target: Multiple

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

Double cortex syndrome (DCS),also known as subcortical band heterotopia, is a congenital brain disorder that arises from aberrant neuronal migration during development of the cortex. It is typically characterized by the appearance of an extra band of gray matter that lies underneath the superficial gray matter of brain cortex and often results in epilepsy and cognitive impairments. The major goal of the proposed research is to establish a human cellular model of DCS to investigate structural deficits in brain formation. We have successfully developed methodology for generating forebrain organoids, three dimensional self-organized structures that can be grown in vitro. These organoids recapitulate a surprising number of features of human embryonic brain development, including the formation of cortical layers. In our study, we have generated induced pluripotent stem cells (iPSCs) derived from a male DCS patient and his unaffected parents, and further generated forebrain organoids from these lines to investigate possible developmental phenotypes. Using multiple approaches including immunostaining and imaging, we characterized the cortical development of organoids over the course of several weeks in culture. During early phases of development, DCS patient-derived organoids showed normal neuronal differentiation, adherens junction and apical basal polarity. At later developing times when the cortical neurons are being generated, we found that patient-derived organoids exhibit differences in the expression of cortical layer-specific markers compared to organoids generated from control iPSCs. We have also observed aberrant morphology and orientation of neural cells in the patient-derived organoids at specific stages of development. Thus, our study strongly suggests that there is a neuronal developmental defect during the formation of cortical structures of patient-derived organoids, which may represent human DCS pathology. In summary, our approach provide a platform to understand cellular mechanisms of DCS and further mechanistic studies may inform the development of novel therapeutic strategies for treatment of this devastating disease.

Marta Lipinski, Ph.D.
University of Maryland, Baltimore
2014 Exploratory Award Budget: $230,000
Disease Target: Parkinson's Disease

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

The goal of our project was to determine the function and mechanisms of the PARK10 gene USP24 in Parkinson's disease (PD). Based on preliminary data we hypothesized that USP24 overexpression and PD-associated polymorphisms contributed to PD by inhibiting autophagy. Conversely, inhibition of USP24 could be neuroprotective and provide a novel treatment against PD. As our PD relevant model we proposed to use long term cultures of human IPSC derived dopaminergic (DA) neurons.We originally proposed to use lentiviral delivery to both knock-down and to express PD-associated USP24 mutants in iPSC neurons. However, since USP24 gene is very large (mRNA in over 8 kbp), we were not able to express it from a lentiviral vector. Therefore, in this work we focused on knock-down of USP24 using lentiviral shRNAs. We are continuing to investigate results of expression of PD associated USP24 mutants using CRIPR technology to mutate endogenous USP24 gene in human cell lines (this work is covered by another grant) and eventually also in human iPSCs. We optimized DA neuronal differentiation of human iPSC neural precursors and obtained 25-30% TH-positive DA neurons. As controls we used lentiviral shRNA to knock-down autophagy regulatory genes (MTOR and BECN1) to increase and decrease levels of autophagy in these cells. We imaged the cells over time during long-term culture (up to 2 months after differentiation) to allow development of .PD relevant phenotypes. Consistent with the importance of autophagy for neuronal survival and health, in aged DA neurons with blocked autophagy we observed fewer/shorter processes and increase in nuclear condensation indicative of cell death. We used lentiviral shRNA to knock-down USP24 in human iPSC derived DA neurons and confirmed that this led to up-regulation of autophagy, supporting our hypothesis that USP24 may contribute to PD via mis-regulation of autophagy. We imaged the cells over time (up to 2 months after differentiation) and quantified PD relevant phenotypes. Our data indicate that neurons with knock-down of USP24 are able to differentiate into general neuronal and into DA phenotypes as efficiently as wild type neurons (either not transduced or transduced with control non-targeting shRNA). USP24 knock-down also did not affect neuronal or DA cell survival over time. Interestingly, in neurons transduced with control shRNA neurite length began to decrease starting 6 weeks after differentiation. On the other hand, neurite length continued to increase in both Tuj1 and DA neurons transduced with shRNA against USP24. This suggests that consistent with our hypothesis, inhibition of USP24 and consequent Increase in autophagy may have beneficial effect on neuronal and DA fitness. We are now getting ready to perform USP24 knock-down in PD patient iPSC derived neurons to determine if USP24 inhibition Is similarly able to increase autophagy and protect them from neurite degeneration.

We also continued our investigation into the mechanisms how USP24 regulates autophagy. USP24 is a deubiquitinating enzyme (DUB) and is predicted to regulate protein stability and activity by removing ubiquitin moieties. Our data place USP24 in the autophagy pathway between mTOR and the type III PI3 kinase. We recently also identified potential targets of USP24 activity. They include ULK1 and Parkin (PARK2). Both proteins are known to positively regulate autophagy and are positively regulated by ubiquitination (ubiquitination increases their stability and/or activity). ULK1 is a protein kinase known to regulate the type III PI3 kinase activity and autophagy downstream of mTOR. Parkin is an ubiquitin ligase necessary for mitophagy and a known familial PD gene. Our data indicate that both ULK1 and Parkin are stabilized in USP24 knock down cells, suggesting that USP24 may normally remove the ubiquitin chains required for their stability and function. We are now confirming the effects of USP24 on the stability, ubiquitination and function of both ULK1 and Parkin in iPCS derived neurons.Our data support our initial hypothesis that USP24 influences PD predisposition by negatively regulating levels of autophagy. Thus USP24 inhibition may be a potential treatment against PD. During the award period we made a substantial progress in both understanding USP24 molecular mechanisms and its function in DA neurons and PD and expect to have a manuscript describing these findings ready for submission by the end of 2016. We are planning to apply for R01 funding for this project in early 2017.

David Nauen, Ph.D.
Johns Hopkins University
2014 Exploratory Award Budget: $230,000
Disease Target: Epilepsy

Investigating Mechanisms of Epileptogenesis Using Human Induced Pluripotent Stem Cells

Our report of the molecular and bioinformatic methodology for sequencing transcriptomes of indiviclual stem cell-derived glioneuronal progenitor cells was published (Shin et al., Cell Stem Cell. 2015 Sep 3; 77 (3):360-72). To increase throughput we have spent considerable effort on optimizing a protocol for dissociation of intact individual brain cells for flow cytometric sorting with exciting recent successes. We have also adapted this method for use on humart tissue with a view to applying it to both patient samples and to experimental samples containing transplanted induced pluripotent stem cell-derived neurons. An excellent new member of our lab is focused on developing methods for identifying mRNAs in inracr tissue specimens, which could be critical in our histological analysis of transplant sections. We have also made progress in synthesis of of a new hydrogel scaffold to improve the effectiveness of the transplantation procedure. We have developed a video-EEG setup with millisecond synchronization between the two data streams and will use this for continuous monitoring to detect seizure development. Collaborators in the Hongjun Song laboratory continue to generate iPSC-derived neuronal progenitor cells and we studied techniques for transplantation of these cells with two experts from that laboratory. We will prepare for the challenging transplantation technique with intrahippocampal injections of a retrovirus, which labels only dividing cells, such that among neurons only those recently born will be labeled. We prepared a report on detection of a source of auto-antibodies that can lead to fatal seizure (in press) and contributed to a report using induced pluripotent stem cell technology to study the mechanisms of Zika virus damage (Qian et al., Cell. 20‘L6May 19;165(5):1238-54).

Tea Soon Park, Ph.D.
Johns Hopkins University
2014 Exploratory Award Budget: $230,000
Disease Target: Diabetic Retinopathy, Ischemic Retinopathy

Treatment of Diabetic Retinopathy with Human iPSC-Derived Vascular Progenitors

Double cortex syndrome (DCS),also known as subcortical band heterotopia, is a congenital brain disorder that arises from aberrant neuronal migration during development of the cortex. It is typically characterized by the appearance of an extra band of gray matter that lies underneath the superficial gray matter of brain cortex and often results in epilepsy and cognitive impairments. The major goal of the proposed research is to establish a human cellular model of DCS to investigate structural deficits in brain formation. We have successfully developed methodology for generating forebrain organoids, three dimensional self-organized structures that can be grown in vitro. These organoids recapitulate a surprising number of features of human embryonic brain development, including the formation of cortical layers. In our study, we have generated induced pluripotent stem cells (iPSCs) derived from a male DCS patient and his unaffected parents, and further generated forebrain organoids from these lines to investigate possible developmental phenotypes. Using multiple approaches including immunostaining and imaging, we characterized the cortical development of organoids over the course of several weeks in culture. During early phases of development, DCS patient-derived organoids showed normal neuronal differentiation, adherens junction and apical basal polarity. At later developing times when the cortical neurons are being generated, we found that patient-derived organoids exhibit differences in the expression of cortical layer-specific markers compared to organoids generated from control iPSCs. We have also observed aberrant morphology and orientation of neural cells in the patient-derived organoids at specific stages of development. Thus, our study strongly suggests that there is a neuronal developmental defect during the formation of cortical structures of patient-derived organoids, which may represent human DCS pathology. In summary, our approach provide a platform to understand cellular mechanisms of DCS and further mechanistic studies may inform the development of novel therapeutic strategies for treatment of this devastating disease.

Feyruz Rassool, Ph.D.
University of Maryland, Baltimore
2014 Exploratory Award Budget: $230,000
Disease Target: Cord blood transplant in blood diseases, Leukemias, Cancers

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

Human induced pluripotent stem cells (hiPSCs), which are reprogrammed from adu lt or progenitor cell types, must make substantial adaptations to become (hESC-like). In this study, we determined whether in vitro conditions that promote efficient reprogramming in hiPSCs also promote an improved genomic integrity that more closely resembles that of hESCs. Studies from the Zambidis (co-PI) group previously demonstrated that human cord-blood (CB) hematopoietic stem progenitor cells were generated at significantly higher efficiency by co-culture on mesenchymal stromal cells (sa CB-iPSCs) (Park TS et al. Plos One 2012). A key difference between these hiPSCs was that sa-CB-iPSCs upregulated MYC-regulated genes, at levels analogous to that of hESC cells referred to as its „MYC signature". In contrast iPSCs generated without MSC co-cu lture i.e. standard CB.iPSCs showed no significant activation of „MYC signature".

Our specific Aims of this proposal were:

I) To determine the mechanism by which MYC regulates the double-strand break (DSB) repair in hESCs and hiPSCs.

2) To determine the role of MYC-regulated DSB repair in efficient generation of hiPSCs.

Translational potential:
Our studies suggest that IPSCs with a MYC expression signature resembling HESCs may be a biomarker for those reprogrammed cells with high genomic integrity and may be used to screen cells for future therapeutic use.

Discoveries from proposed Aims:
Discovery 1: CB progenitors and CB-derived iPSCs closely resemble hESCs in DNA repair gene expression signature. The DNA damage response (DDR) in non-integrated episomal iPSCs derived from (sa-CB-iPSC) were compared with DDR in standard derived CB-iPSCs, in fibroblast derived iPSCs and compared this response to hESCs (N =3 each). We therefore first performed microarray analysis for DNA single-strand and DSB repair genes in these cells and verified by quantitative PCR and Western blotting analysis that both CB.iPSC I ines have strong gene expression similarities to hESCs. Interestingly, donor CD34+ CB progenitors cluster more closely with hESCs than adult fibroblasts (Ad.Fib) donors in baseline expression of DNA repair genes. These results suggested that CD34+ CB progenitors may already possess hESC-like expression of DDR pathway components, even prior to initiation of reprogramming.

Discovery 2: Sa-CB-iPSCs resemble hESCs in their DDR response to radiation. Irradiation (IR) elicits several post translational modifications of the components of DDR pathway. To determine the efficacy of DDR, representative CB derived hiPSCs (i.e., sa-CB-iPSC (CB6.2), standard CB-derived hiPSC (iCB9) and fibroblast-derived (iHUF3) were treated with IR (2Gy), and compared with IR-treated hESCs (i.e., H9 and ES03). Our results suggest that radiation induced DNA damage checkpoints and apoptosis proceeded with similar kinetics in all tested hESCs and hiPSCs. Thus, reprogramming renders all hiPSCs equally hypersensitive to ionizing radiation-induced apoptosis.

Discovery 3: Sa-CB-iPSCs more closely resemble hESCs in non-homologous end-joining (NHEJ) repair. Functional assays for double-strand break (DSB) end-joining repair demonstrated that sa-CB-iPSC and hESCs exhibited similar levels of repair accuracy. In contrast, standard CB-iPSC and the I ess efficiently generated Fib-iPSC not only exhibited Jess efficiency ofNHEJ but also more error-prone NHEJ activities.

Discovery 4: C-MYC maintains the DDR and NHEJ in hESCs and is required for less error-prone repair in sa-CB iPSCs. MYC modules, along with Core and Polycomb group genes, represent key gene circuits that contribute to the ES cell expression signature. C-MYC depletion from the reprogramming cocktail significantly reduces the efficiency of reprogramming. Notably, compared with untreated cells, C-MYC inhibition results in increased level of DSB markers that persist for prolonged duration. This data indicates that C-MYC is involved in the radiation-induced DSB repair response in hESCs, facilitating repair. "Error-proness" of NHEJ si gnificantly escalates when pluripotent cells are subjected to IR stress under conditions of MYC inhibition. Moreover, analysis of DSB repair junctions indicated that the efficacy of DNA end-joining significantly deteriorated and became more error-prone when C-MYC was depleted in hiPSCs. These results imply that C-MYC gene expression signature is linked to efficacious NHEJ DSB repair in pluripotent cells.

In conclusion, our studies show that the various methods for generating hiPSCs may affect the pathways that regulate genomic integrity. Further characterization is required to determine how these pathways are interconnected and will enable improvement of the genomic integrity of hiPSCs. Knowledge that C-MYC is also a master regulator of chromatin modifications, its role in facilitating repair might not only be transcriptionally regulated but also epigenetically controlled. Thus, further elucidation of the role of C-MYC in maintenance of genomic integrity, regulating the balance between "good repair" vs. "bad repair" in pluripotent cells is required.

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Disease Target: Hutchinson-Gilford Progeria Syndrome

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

We have generated mesenchymal stem cells (MSCs) from iPSCs isolated from patients with Hutchinson Gilford Progeria Syndrome (HGPS) and sibling controls. Furthermore, we have established the conditions for differentiating these iPSCs derived MSCs into bone forming osteoblasts, by supplementing with a cocktail of dexamethasone, ascorbic acid and beta-glycerophosphate. We have demonstrated that these cells successfully form mineralized nodules in vitro using Alizarin Red staining. Notably. HGPS-derived cells displayed decreased mineralization relative to normal control derived osteoblasts. In parallel. we have used human bone marrow derived MSCs overexpressing wild type lamin A or progerin to model HGPS. In this system, we have shown a significant difference in the mineralization between controls, with a marked reduction in mineralizing capacity in cells overexpressing progerin. These data are consistent with the decreased bone forming activity in patients with HGPS.

Consistent with our hypothesis, both iPSC derived and BMSC models showed that progerin/HGPS resulted in reduced active beta-catenin levels, supporting our hypothesis that abberant beta-catenin signaling underlies the skeletal phenotype observed in HGPS. Preliminary experiments suggest that this is due to altered by the farsenylated progerin protein.as a mutant non-farnesylated progerin protein does not restrict active beta-catenin levels.

Using Zmpste24-/- mice as a model for HGPS. we have shown by micro-computed tomography that these mice have a severe osteopenic phenotype relative to age and gender matched control mice (Zmpste24+/+). We observed statically significant decreases in trabecular bone and cortical bone in these mice. Western blots of bone extracts reveal decreased beta-catenin signaling in the Zmpste24-/ mice (as was observed in the HGPS iPSCs).Administration of an anti-sclerostin antibody. which de represses beta-catenin signaling, improves bone mass in both male and female Zmpste24+/+ mice. Importantly, the anti-sclerostin antibody is extraordinarily effective in the Zmpste24-/- mice, fully restoring bone mass (cortical and trabecular) in these animals. This data strongly suggests that defective Wnt-beta-catenin signaling is fundamentally important to the skeletal phenotype in HGPS. Accordingly, administration of this therapy (anti-sclerostin antibodies- which are in phase 3 clinical trials) could be beneficial to patients with HGPS. Furthermore, while our intervention was bone specific, the fundamental nature of Wnt/beta-catenin signaling to stem cell renewal and tissue repair and regeneration makes this a viable candidate pathway for pathologies in other tissues in HGPS. Manuscripts detailing this work are underway.

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