MARYLAND
STEM CELL RESEARCH FUND
ANNUAL REPORT

2013

MSCRF
Maryland Stem Cell Research Fund

Promoting State Funded Stem Cell Research & Cures

On behalf of the Maryland Stem Cell Research Commission and the Maryland Technology Development Corporation, we are proud to present to you the **Seventh Annual Maryland Stem Cell Research Report**.

We are fortunate to have the ongoing support of Governor Martin O'Malley, Senate President Miller, House Speaker Busch and the members of Maryland legislature, who recognize the potential of stem cell research scientifically and medically, and as an economic engine for the State of Maryland. Their vision allows Maryland to remain at the forefront of this promising new medical frontier.

One of our priorities has always been to educate the public on the importance of stem cell research This Annual Report provides an opportunity to showcase our research that we hope facilitate collaboration and promote best practices. And, of course, this affords all of us the chance to recognize the wonderful work that is being done locally. This is an exciting time and an exciting venture on which we are all embarked and which I am grateful to have a small role in -- being unable to do the heavy scientific lifting that most of you do.

The MSCRF was created in 2006 pursuant to the MD Stem Cell Research Act and has in its short time in existence given out over $100 million in grants to some 300 different research projects.

During that time we have sponsored research which impacts multiple diseases: Parkinson's disease and Huntington's Disease, Alzheimer's, Crohn's, Gaucher's and ALS, known as Lou Gehrig's Disease, Heart damage, Brain damage, Bone damage, Spinal Cord damage, Skin damage, healing wounds , Arterial disease, Kidney disease, Liver disease, Sickle Cell disease, Retinal disease as well as, Multiple Sclerosis (MS), Diabetes, Stroke Autism, Down's Syndrome, and Schizophrenia. Multiple studies have focused on various cancers - Cancers of the brain, breast, lung, pancreas and blood, among others.

Some of these projects have had small yet meaningful successes that were featured in many scientific and other media publications.

Here it should be said that the commission has articulated a guiding principle that our funding is concerned with advancing cell therapy, thus we are interested in funding research on repair of cancerous stem cells and not their destruction. We have focused our attention on the generation of and recognition of stem cells, their regulation and manipulation, the path to their differentiation into specific tissues and the potential regeneration of disease free tissue.

Sincerely,

Rabbi Avram I. Reisner, Ph.D.
Chair
Maryland Stem Cell Research Commission

Robert A. Rosenbaum
President and Executive Director
Maryland TEDCO

MARYLAND STEM CELL RESEARCH COMMISSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2013 GRANT AWARDS

Clincal & Pre-Clincal Grant Award

Lew Schon
The Stem Cell Suture Company
In Collaboration w/ MedStar Health Research Institute & Union Memorial Hospital
"Stem Cell Bearing Suture for Tendon/Ligament Reconstruction in Healing Impaired Patients"

Investigator Initiated Grant Awards

Angelo All, M.D., M.B.A
Johns Hopkins University (JHU)
In collaboration w/ University of Maryland, Baltimore
"Acute Hypothermia and Induced Human Oligodendrocyte Precursor Cell Transplants for Improve Functionality after Spinal Cord Injury"

Robert Brodsky, M.D.
Johns Hopkins University (JHU)
"BMT for Sickle Cell Disease and Lupus"

Valina Dawson, Ph.D.
Johns Hopkins University- School of Medicine (JHU)
"Parkinson's Disease Causing Mutations in LRRK2 Lead to Neuronal Dysfunction in Human Neurons"

Assaf Gilad, Ph.D.
Johns Hopkins University (JHU)
In Collaboration with Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
"Visualization of Gene Expression Promoting Adipose Derived Mesenchymal Stem Cell Tropism For Brain Tumors"

Yoon-Young Jang, M.D., Ph.D.
Johns Hopkins University (JHU)
In Collaboration w/ Duke Liver Center at Duke University & California Institute for Regenerative Medicine (CIRM)
"Developing Novel Therapy for Liver Fibrosis Using Patient-Specific Stem Cells"

Sunjay Kaushal, M.D.
University of Maryland, Baltimore (UMB)
"Characterization of Resident Cardiac Stem Cells in Neonates"

Gabsang Lee, Ph.D.
Johns Hopkins University (JHU)
In Collaboration with Life Technologies Corporation
"Direct Conversion of Fibroblasts into Induced Neural Crest (iNC) With Single Transcription Factor & Cell Extrinsic Factors"

Saul Sharkis, Ph.D.
Johns Hopkins University (JHU)
"Stem Cell Transplantation Therapy for Diabetes"

Leslie Tung, Ph.D.
Johns Hopkins University (JHU)
In Collaboration with National Institutes of Health (NIH)
"Mechanistic Studies of ARVD/C Dysfunction Using Human iPSC-derived Cardiac Tissues"

Michal Zalzman, Ph.D.
University of Maryland (UMB)
"Rejuvenation of Human Adult Mesenchymal Stem Cells for Clinical Applications"

MSCRF
Maryland Stem Cell Research Fund

2013 GRANT AWARDS

Exploratory Grant Awards

Jeff Bulte, Ph.D.
Johns Hopkins University (JHU)
In Collaboration w/ Cosmeticsurg & University of Maryland
"19F Hot Spot MRI of Human Adipose-Derived Stem & Progenitor Cells for Breast Reconstruction"

Kimberly M. Christian, Ph.D.
Johns Hopkins University (JHU)
"Using Human Neurons for Functional Reorganization in a Model of Temporal Lobe Epilepsy"

Hugo Guerrero-Cazares, M.D., Ph.D.
Johns Hopkins University (JHU)
"Controlling Migration of Human-Derived Fetal Neural Stem Cells Via Slit Proteins in a Demyelination Animal Model"

Xiaofeng Jia, M.D., Ph.D.
Johns Hopkins University(JHU)
"Electrical Stimulation on Neural Crest Stem Cell Transplantation in Nerve Regeneration"

HanSeok Ko, Ph.D.
Johns Hopkins University (JHU)
"Characterization of Glucocerebrosidase Deficiency Pathway in Parkinson's Disease"

Eugene Koh, M.D., Ph.D.
University of Maryland, Baltimore (UMB)
In Collaboration w/ Pearl Biosciences, LLC
"Identification of Small Molecules to Direct Mesenchymal Stems Cells Differentiation into Intervertebral Disc Chondrocytes"

Seulki Lee, Ph.D.
Johns Hopkins University (JHU)
In Collaboration w/ Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
"Design of Highly Fluorinated Stem Cells for 19F MR Imaging in Cardiac Repair"

Pablo Sanchez, M.D.
University of Maryland (UMB)
In Collaboration w/ The Living Legacy Foundation & XVIVO Perfusion
"Bone Marrow Derived Stem Cells to Improve Donor Lung Quality & Transplant Outcomes"

Matthew Trudeau, Ph.D.
University of Maryland (UMB)
In Collaboration with Paragon Bioservices, Inc.
"Potassium Channels and Human Cardiomyocytes Derived from Stem Cells"

Kathryn Wagner, M.D., Ph.D.
Hugo W. Moser Research Institute at Kennedy Krieger
In Collaboration w/ Johns Hopkins University (JHU)
"A Three Dimensional Environment for Skeletal Muscle Stem Cell Transplantation"

2013 GRANT AWARDS

Post Doctoral Fellowship Grant Awards

Akshata Almad, Ph.D.
Johns Hopkins University (JHU)
"Human iPSC Cell-Derived Astrocytes to Study ALS & Astrocyte Connexins as a Therapeutic Target"

Peter Andersen, Ph.D.
Johns Hopkins University (JHU)
"Identification of chamber-specific Cardiac Progenitor Populations"

Jing Cai, Ph.D.
Johns Hopkins University(JHU)
"Hippo Signaling In Intestinal Stem Cell Homeostasis & Carcinogenesis"

Fabien Delaspre, Ph.D.
Johns Hopkins University (JHU)
"Maturation of Human Embryonic Stem Cells-Derived Pancreatic Progenitors into Insulin Producing Cells"

Jing Fan, Ph.D.
Johns Hopkins University (JHU)
"PARP-1 and Histone1 Interplay and Regulate Stem Cell Differentiation after Stroke"

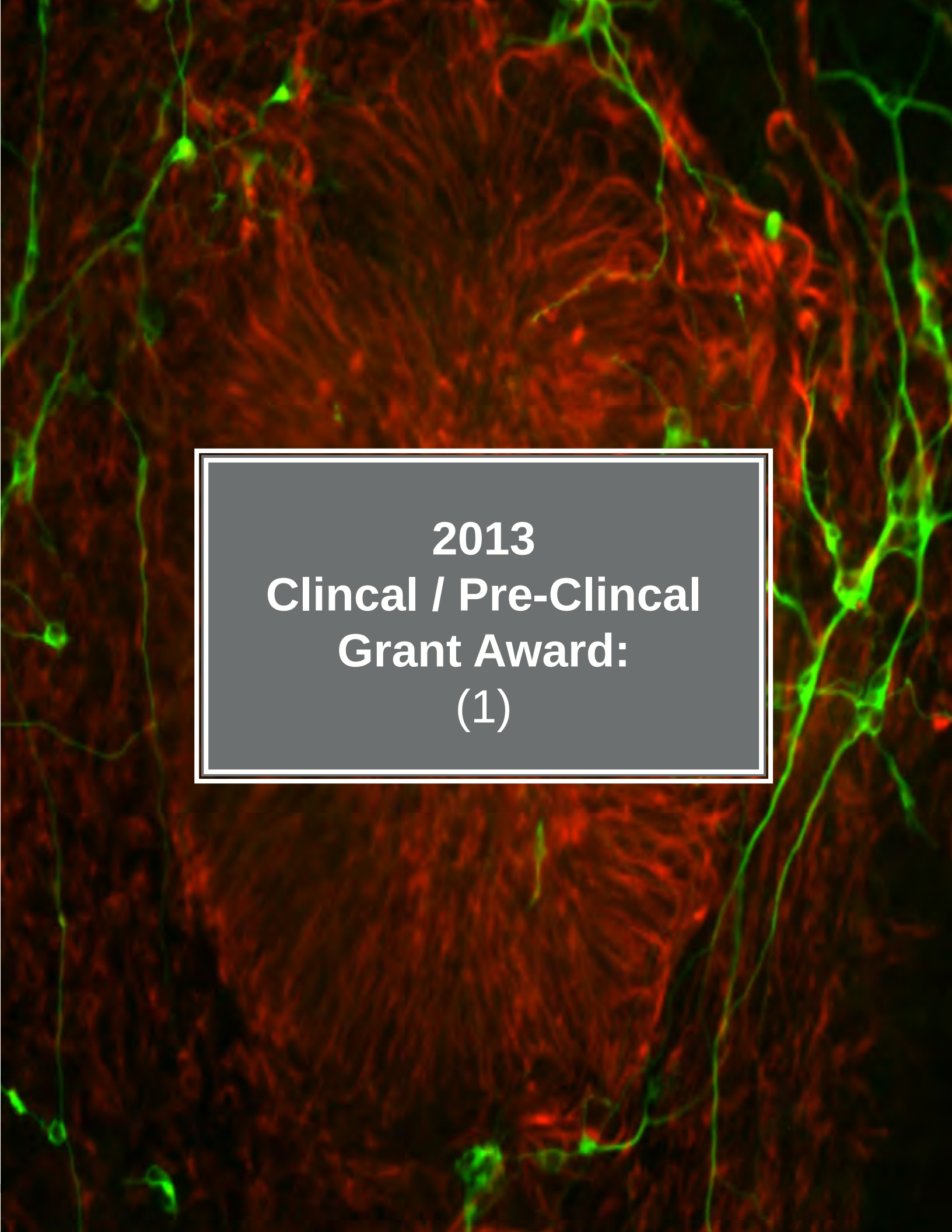
Jeffrey Huo, M.D., Ph.D.
Johns Hopkins University (JHU)
"The Role of Somatic Memory in Determining Efficient Hematopoietic Differentiation of hiPSC"

Xiaowei Li, Ph. D.
Johns Hopkins University (JHU)
"Enhancing Therapeutic Potential of Human Neural Stem Cells"

Anjali Nandal, Ph.D.
University of Maryland, College Park (UMCP)
"Induced Pluripotent Stem Cell Derived, Immunoisolated Î²-cell Transplantation for Diabetes Therapy"

Jinchong Xu, Ph.D.
Johns Hopkins University (JHU)
"Cell Replacement for Stroke Using hiPSC Derived 3D-Organized Cortical Neurons"

Ludovic Zimmerlin, Ph.D.
Johns Hopkins University (JHU)
"Genetic correction of Sickle-Cell-Disease Human iPSC Converted To a Murine-ESC-Like State"



2013
Clincal / Pre-Clincal
Grant Award:
(1)

Lew Schon
The Stem Cell Suture Company
Awarded Budged: $559,000.00
Disease Target: Rheumatoid Arthritis

STEM CELL BEARING SUTURE FOR TENDON/LIGAMENT RECONSTRUCTION IN HEALING IMPAIRED PATIENTS

Mesenchymal stem cells (MSC) play a fundamental role in structural tissue repair. One of the reasons healthy young individuals generally heal well from injury or surgery is that they have numerous MSCs.On the other hand, as a person ages, the number of MSC produced dramatically decreases and theses cell's helpful participation in repair and recovery is limited. In addition, in many chronic conditions such as autoimmune and other systemic diseases, healing can be further compromised. Thus, older individuals especially those with complicating conditions who develop musculoskeletal disorders such as tendon ruptures, joint destruction from arthritis or ligament failure are at an increased risk of surgical failure and long-term (permanent) disability with a corresponding impact on quality of life and financial well-being. Elevated concentrations of stem cells at the site of desired tissue regeneration are important for orchestrating tissue repair, helping to modify inflammation and improving circulation. Though injection of very large numbers of stem cells into the area of desired tissue repair is thought to provide benefit, the FDA has indicated concern that injected stem cells have the potential to migrate to other parts of the body with unknown effect in these other sites. There is research that indicates that injected stem cells tend to lodge in the microvasculature of the organs, such as the lungs, kidneys and liver. This project's technology provides the surgeon with a densely loaded, stem cell bearing scaffold (suture) which can be used with conventional surgical techniques to provide compromised individuals with the benefits of targeted delivery of stem cells while reducing the potential for unwanted migration of the cells to the critical organs in the body. Our research indicates that delivering the stem cells in our scaffold can provide improved benefits over direct injection in the form of healthier tissue, reduced scar tissue formation and reduced stem cell migration. Additionally, our preliminary studies indicate that our technology may be effective in dramatically increasing surgical success in cases with complicating chronic diseases. The technology provides both a unique scaffold and a carefully selected subset of Mesenchymal stem cells. The current project is directed towards the pre-clinical trial work necessary to quickly move this technology into clinical trials as an orphan drug, initially benefiting individuals at elevated risk of surgical failure in tendon/ligament repair and joint replacements due to a chronic disease complication. This is a technology which can both improve quality of life as well as reduce the incidence and cost of long-term disabilities and should be of interest to those who want to remain active in their later years.

2013 Investigator Initiated Grant Awards:

(10)

Angelo All, M.D., M.B.A
Johns Hopkins University, (JHU)
In collaboration w/University of Maryland, Baltimore
Awarded Budged: $618,290.00
Disease Target: Spinal Cord Injury

ACUTE HYPOTHERMIA & INDUCED HUMAN OLIGODENDROCYTE PRECURSOR CELL TRANSPLANTS FOR IMPROVED FUNCTIONALITY AFTER SPINAL CORD INJURY

Spinal cord injury (SCI) causes severely decreased mobility and consequently lead to a poor quality of life for those suffering from SCI. The mechanism of injury has two phases. The second phase involves the death of a type of cell called oligodendrocytes (OLs) within the spinal cord. These cells provide myelin, the electrical insulation around neurons that is necessary for efficient signal conduction to and from the brain. Hence, one approach to treat SCI is to replace these OLs with stem cell-derived myelinating cells in patients. We propose to do this in a novel, patient-specific way and in conjunction with hypothermia treatment, which has previously been shown in our lab to limit secondary injury when applied within two hours of injury. We propose to directly convert adult human cells into oligodendrocyte progenitor cells, which is one step before mature oligodendrocytes (OLs) cells. This is done by using forced expression of transcription factor Sox2, using a modified 1-factor reprogramming strategy to derive functional oligodendrocyte progenitors (Sox2-transduced i-hOP cells) from adult human fibroblasts. Adding to this one transcription factor (Sox2), the correct growth factors and cytokines known to induced OL fate will lead to the loss of fibroblast identity and if expressed for the right amount of time, these cells do not become pluripotent and instead are directly converted to induced human oligodendrocyte progenitors called Sox2-transduced i-hOP cells. This bypassing of the pluripotent stage is the innovation of our proposal and differentiates it from other methods which use induced pluripotent stem cell or embryonic stem cell lines which have immunological and ethical concerns. Our proposal builds upon our previous study which showed that acute moderate hypothermia applied two hours after SCI, led to a neuroprotective effect which lasted for four weeks. This implies that onetime administration of 2 hour acute hypothermia is sufficient to provide a month long window of opportunity in which to administer a permanent treatment to the patient. The i-hOP cells can be generated within four weeks and hence the study design is to apply local moderate hypothermia immediately after injury, followed by a cell transplant four weeks later. The combination of these two therapies is expected to lead to significant recovery as monitored by electrophysiology, motor behavior scoring and myelination of spared axons, studied by electron microscopy. The significance of this project lies in the design of a highly effective and patient-specific clinical therapy for human SCI.

Robert A. Brodsky, M.D.
Johns Hopkins University, (JHU)
Awarded Budged: $690,000.00
Disease Target: Sickle Cell Disease & Lupus

BMT FOR SICKLE CELL DISEASE & LUPUS

This is a high impact proposal using adult hematopoietic stem cells in an attempt to cure two highly underfunded diseases (sickle cell anemia and lupus) that primarily affect underrepresented minority populations in Maryland. Sickle cell disease (SCD) is a genetic disease that predominantly affects African Americans in the United States (US) and is associated with reduced quality of life and premature mortality. The annual cost of medical care in the US for people with SCD exceeds $1 billion dollars. Systemic lupus erythematosus (SLE) is a devastating systemic autoimmune disease that predominantly affects young women, is more common in African-Americans than in whites, and also results in poor quality of life. Genetic diseases (e.g, SCD) and autoimmune diseases (e.g, SLE) are prime targets for regenerative medicine. Embryonic stem (ES) cells and induced pluripotent stem cells (iPSC) have promise, but safety concerns and the fact that hematopoietic stem cells (HSC)derived from ES and iPSC are not yet capable of long-term engraftment limits their potential for regenerative medicine in blood diseases such as SCD or SLE. Adults stem cells derived from blood or marrow may also be used for regenerative medicine. Their major limitation has been the inability for most patients to find a perfectly matched donor and complications of the transplant such as graft-versus-host disease (GVHD). We have developed a novel stem cell transplant procedure that uses low dose chemotherapy and is so effective at eradicating GVHD that we no longer require matched sibling donors; half-matched donors are equally effective. Half-matched related donors are easy to find; 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. Using this approach, our preliminary data suggest that most patients with SCD can be cured using this approach; furthermore, we show that lupus and other severe autoimmune diseases may be cured. This innovative work was recently featured as the Plenary Article in the hematology journal Blood and The New York Times. Thus, the overall goals of this proposal are: 1) to improve the cure rate of SCD using adult stem cells and 2) to develop, for the first time, a curative therapy for SLE using adult stem cells. Importantly, these trials have already started; thus, there is high likelihood for success and translation to the clinic. Moreover, continued success could change paradigms for treating and understanding SCD and SLE.

Valina Dawson, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $690,000.00
Disease Target: Parkinson's Disease

PARKINSON'S DISEASE CAUSING MUTATIONS IN LRRK2 LEAD TO NEURONAL DYSFUNCTION IN HUMAN NEURONS

Parkinson's disease (PD) is a progressive neurodegenerative disorder that profoundly affects movement. In PD the loss of neurons, and in particular dopamine (DA) neurons that are important for regulating motor function result in the debilitating symptoms experienced by patients with PD. The onset of sporadic PD is noticeably related to aging, with a sharp rise in incidence seen after the age of 60. The cause of most cases of PD is unknown but there are rare families in which PD is inherited. Identification of the genes that are mutated in these families and the study of the changes in cell function that occurs due to these mutations has allowed significant advances in understanding the mechanisms of disease pathogenesis and the identification of potential new therapeutic targets. Gene mutations in leucine-rich repeat kinase 2 (LRRK2) lead to PD families in which PD is inherited but there is also a high prevalence of LRRK2 mutations in sporadic PD patients. Of the various mutations in LRRK2 the LRRK2 G2019S is the most common and when this mutant is expressed in worms, flies or mice it leads to dopaminergic neurodegeneration. We have learned this disease causing mutation leads to changes in protein expression and propose to use human dopamine neurons to understand how LRRK2 changes protein expression and to learn how to use this information to identify new drug opportunities for the treatment of PD.

Assaf Gilad, Ph.D.
Johns Hopkins University, (JHU)
In Collaboration w/ Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
Awarded Budged: $679,049.00
Disease Target: Brain Cancer

VISUALIZATION OF GENE EXPRESSION PROMOTING ADIPOSE-DERIVED MESENCHYMAL STEM CELL TROPISM FOR BRAIN TUMORS

Stem cells research revolutionized modern medicine and offers new directions to treat many incurable diseases. Therapeutic stem cells, or the idea of taking stem cells from a donor or from the patient her/himself has been developed mostly for curing degenerative disease. However, a great potential lays hidden in the ability of stem cells to cure cancer, specifically, incurable types like glioblastoma (GB). Interestingly, many stem cells have a natural tendency to chase and track down tumors. This is because tumors transmit signals (in the form of soluble proteins) that are picked up by receptors on the stem cells membrane. This is not surprising, as tumors usually abuse and enhance existing systems that are used in natural physiological process such as embryonic development and wound healing. Therefore, many studies have proposed that by engineering stem cells it will be possible to enhance the natural tendency of these cells to migrate toward tumors. Nevertheless, the lake of appropriate imaging tools limited the understanding of the mechanisms governing these processes, and prevented the translation of this novel concept into a practical treatment.With an overall goal of improving cancer patients' well-being as well as treatment safety in mind, we have decided to use human adipose-derived mesenchymal stem cells (hAMSCs). These cells are naturally found in fat tissue in the patient's own body and are purified and enriched from the patient her/himself. The advantages of using such cells are numerous, including biocompatibility with the patients (i.e., will not activate the immune system), purification from a tissue that would be otherwise discarded, and most importantly, they do not require destruction of live embryos. Our laboratory specializes in developing biosensors for non-invasive imaging of biological process. Specifically, we are using these probes for real-time monitoring of gene expression and tracking cells in the body. Here we propose to capitalize on innovative imaging technologies that were developed in our laboratory in order to visualize the homing of hAMSCs toward brain tumors. We assembled a team of experts in cutting edge imaging technologies, stem cells biology and brain cancer to (1) Determine the role of soluble factors that drive stem cells toward the tumor and (2) Engineer the hAMSCs to express more of the receptors for those factor and thus, improving their migration toward the tumor. In the future, these cells can be used to carry therapeutic cargo to tumors in order to track down malignant tumor cells and metastasis. In this study, engineered stem cells isolated from adult patients will be used together with state-of-the-art imaging technologies to study and understand their interactions with brain tumors. Once accomplish, these findings could be translated into the clinic in order to enhance and improved personalized medicine.

Yoon Young Jang, M.D., Ph.D.
Johns Hopkins University, (JHU)
In Collaboration w/ Duke Liver Center at Duke University & CIRM
Awarded Budged: $690,000.00
Disease Target: Liver Fibrosis

DEVELOPING NOVEL THERAPY FOR LIVER FIBROSIS USING PATIENT-SPECIFIC STEM CELLS

The overarching goal of this study is to develop stem cell models for promoting liver regeneration and preventing damages in alcoholic liver fibrosis which is the most common cause of liver cirrhosis in the United States and is associated with a substantial economic burden. There is currently no effective therapy for liver cirrhosis, largely due to a lack of human cell-based model systems to study this disease and to develop treatment strategies. In order to overcome this hurdle, we have successfully generated multiple patient-specific induced pluripotent stem cell (iPSC) lines from liver cirrhosis patients for modeling this disease. By directed differentiation of these iPSCs into hepatic cells, we have recapitulated the fibrotic disease feature in a dish. Through comparative molecular analysis of these cells, we have also identified candidate genetic variants that may play important roles in liver fibrosis. Although some of these variants have been shown in genome-wide association studies to strongly associate with susceptibility to alcoholic cirrhosis, there have been few viable strategies to determine their biological roles in disease progression. Here we propose to use our iPSC model and the latest genome editing technology to functionally evaluate these genetic variants. Completion of this aim will not only gain insight into disease mechanisms, but also identify molecular targets for developing diagnostic and therapeutic strategies. A second aim of this project is to develop an iPSC-based drug screening platform to discover potential drugs that may reduce or prevent liver fibrosis. To expedite the application of lead compounds to patients, we will conduct screening utilizing a library of clinical drugs with extensive safety profiles, as we have recently done for another liver disease. In summary, completion of this study will significantly enhance our understanding of molecular mechanisms underlying liver fibrosis/cirrhosis and provide new therapy for the cirrhosis patients without therapeutic options. In addition, it will provide a broadly applicable and revolutionary platform for utilizing the existing (and increasingly abandoned) genomic data for both basic and translational studies including developing biomarkers for many human diseases.

Sunjay Kaushal, M.D.
University of Maryland, Baltimore (UMB)
Awarded Budged: $690,000.00
Disease Target: Heart Failure

CHARACTERIZATION OF RESIDENT CARDIAC STEM CELLS IN NEONATES

Heart failure is a common and devastating condition that leads to death. Common clinical situations, such as high blood pressure or coronary artery disease, leads to heart attacks which are the major causes of heart failure. Although there have been significant advances in the treatment of heart failure in recent decades, there is still a major unmet need for better treatments for many patients with heart failure, especially the very young. Available treatments do not address the fundamental problem of the loss of cardiac tissue. As a result, interest in attempts to repair the failing heart with the use of stem cells has been increasing, since this approach has the potential to regenerate dead myocardium and thus alleviate the underlying cause of heart failure. Recent evidence from our laboratory and others' has identified a population of cells within the heart itself, cardiac stem cells, which may have the unique ability to stimulate heart muscle cell growth (regeneration). Cardiac stem cells have the potential to be reimplanted in the same individual they come from and improve their cardiac function. We propose to understand the basic biology of these stem cells in the very young and old-two populations that may benefit from this type of treatment. This project will be an important first step in generating pre-clinical laboratory data to determine whether this can be a promising therapy for these unfortunate heart failure patients.

Gabsang Lee, Ph.D.
Johns Hopkins University, (JHU)
In Collaboration w/Life Technologies Corporation
Awarded Budged: $689,999.00
Disease Target: Multiple

DIRECT CONVERSION OF FIBROBLASTS INTO INDUCED NEURAL CREST (iNC) WITH SINGLE TRANSCRIPTION FACTOR & CELL EXTRINSIC FACTORS

Peripheral neuropathies affect patients who have genetic mutations, neurodegenerative diseases, diabetes, chemotherapy treatment, alcohol abuse experience, physical trauma and HIV infection, which result in uncountable economic burden in our society. Currently most of the study related with peripheral neuropathies is based on rodents that often present irrelevant phenotypes and results than that of humans, which emphasizes the development of new human system. Understanding human peripheral neuropathies has been hardly possible, mainly due to scarcity of patient samples.
Recently our lab created a novel methodology of coaxing human skin cells into peripheral neuronal precursors. By comparing our accumulated expertise with human pluripotent stem cells over 8 years, this new approach shows a new promising direction in terms of rapid generation of peripheral neuronal precursors, employing minimal genetic manipulation and individualized derivation. We believe that those advantages lead us to accelerate its application into translational study, including disease modeling of human neuropathies, personalized drug discoveries and autologous cell transplantation.

Saul Sharkis, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $690,000.00
Disease Target: Diabetes Mellitus

STEM CELL TRANSPLANTATION THERAPY FOR DIABETES

The use of adult stem cells for regeneration/repair of injured tissue has been demonstrated using both animal and human stem cells. Bone marrow transplants have been used for many years for treatment of hematological malignancies, bone marrow failure conditions and other hematological and autoimmune disorders. We have demonstrated in mice that stem cells isolated from the bone marrow by functional selection appear more primitive than stem cells selected by phenotypic markers. In order to utilize the most efficacious stem cell for repair of injured or diseased tissue it is necessary to select the most primitive and most multipotent stem cell for the repair. We plan to isolate human bone marrow derived stem cells (hBMSC) to establish the purity and potency of these cells so they may be used for regenerative medical therapy. We will perform limiting dilution analysis of the selected cells and competitive repopulation against phenotypically isolated stem cells to establish the engraftment potential (purity) and differentiation/self renewal potential (potency) of the functionally isolated cells. Thus our first specific aim is to isolate primitive hBMSC for the purpose of using these cells in cellular clinical therapy. Once we have established that our methods of selection provide an early hBMSC we will attempt to treat 2 models of Diabetes in immunodeficient recipient mice with these cells to repair injury to epithelial tissue (i.e. Beta cells) of the pancreas. We will use chemical destruction of the pancreatic cells which produce insulin which will mimic the condition of Diabetes in patients. Diabetes is a global problem with both severe physical and economic damage. In the United States alone over 20 million people are affected. In Maryland 5-7 % of the public is affected by this disease. The disease results from loss of functioning Beta cells of the pancreas. We have data the indicates that rare multipotent stem cells can reverse the metabolic damage associated with Diabetes and in this proposal plan to demonstrate that transplantation of purified human stem cell populations can regenerate Beta cells and correct the metabolic glucose dysfunction. Thus we will either treat immunodeficient NSG mice with Streptozotocin which chemically destroys Beta cells of the pancreas or diabetic mutationally altered mice with stem cells. Animals will be monitored for 6 months for Blood Glucose levels, Glucose Tolerance Tests and Body Weight changes. The mice will be sacrificed and the pancreatic, liver and hematopoietic tissue examined for evidence of functional human cells which in the pancreas produce insulin. If conversion of one or more human stem cell populations produce functional Beta cells we can conclude that these stem cells can be used for transplant therapy.

Leslie Tung, Ph.D.
Johns Hopkins University, (JHU)
In Collaboration w/National Institutes of Health (NIH)
Awarded Budged: $684,930.00
Disease Target: Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C)

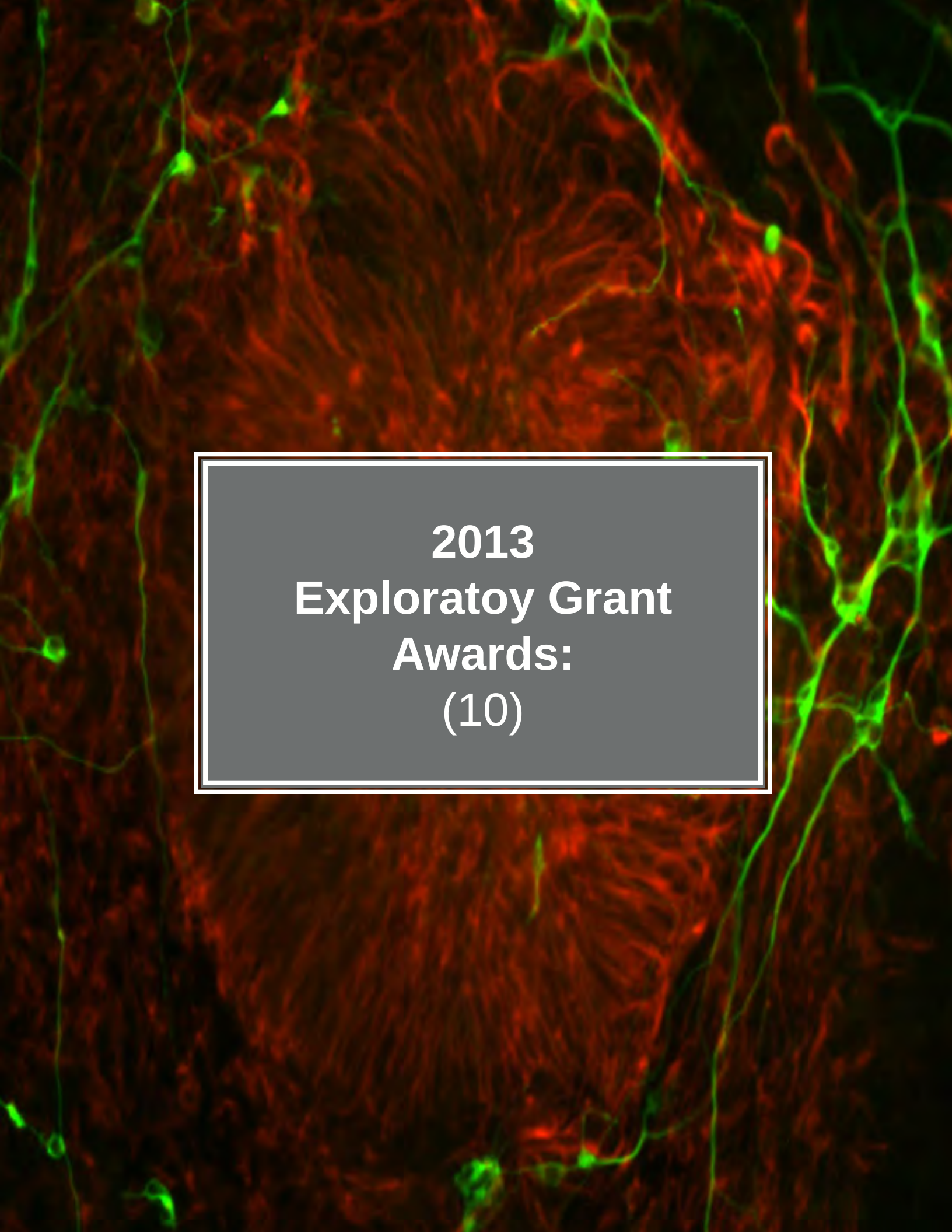
MECHANISTIC STUDIES OF ARVD/C DYSFUNCTION USING HUMAN IPSC DERIVED CARDIAC TISSUES

Exciting progress has been made in the reprogramming of blood and skin of human donors into heart cells for the development of new models of cardiac disease never before possible. The derivation of cardiomyocytes from human induced pluripotent stem cells (hiPSC-CMs) is now relatively well established, and has opened up the very exciting possibility for the creation of in vitro human models of inheritable cardiac diseases. In this project we will create tissue models for one genetic cardiac disorder (arrhythmogenic right ventricular dysplasia/cardiomyopathy) that adversely affects electric currents in heart to cause sudden cardiac death. This project is a collaborative effort between basic scientists and clinicians at the Johns Hopkins University and Johns Hopkins Hospital. We will engineer precision cut, human heart slices that manifest the cardiac disease, arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), an inherited cardiomyopathy characterized by frequent and severe arrhythmias and sudden cardiac death. This will be achieved by highly efficient differentiation of cardiomyocytes from iPSCs generated from ARVD/C patients, and seeding the cells on native tissue matrix, which will provide a natural extracellular protein and fiber structure to support the growth and electromechanical coupling of hiPSC-CMs. This innovative approach, which has been successful with non-human derived CMs, will result in electrophysiologically and mechanically functional tissues that retain hallmarks of the cardiac disease. Rigorous electrophysiological assays and complementary histological and molecular analysis will then be performed on these reconstructed human tissue samples. The proposed work would be the first to model these currents among cells in three dimensions in the laboratory to mimic the native disease process. These models will be used to better understand factors that predispose to arrhythmia, and tests of potential therapies for treatment. The specific aims of the project are: (1) to create a working tissue model (engineered heart slice, EHS) of ARVD/C using cardiomyocytes derived from human induced pluripotent stem cells derived from human donors, and (2) to utilize the EHS model of ARVD/C to test putative mechanisms of arrhythmia. In summary, This is a critical step towards the realization of the treatment and management of this disease, and success will establish a paradigm for the analysis and treatment of other human heart arrhythmia syndromes in the laboratory.

Michal Zalzman, Ph.D.
University of Maryland, Baltimore (UMB)
Awarded Budged: $690,000.00
Disease Target: Multiple

REJUVENATION OF HUMAN ADULT MESENCHYMAL STEM CELLS FOR CLINICAL APPLICATIONS

Human stem cells possess the ability to develop into a wide range of specialized human tissues, such as nerves, muscle, bone, kidney and lung. Mesenchymal stem cells (MSC) are a subset of human stem cells that can be harvested from adults and grown in the laboratory with the potential for use in replacing cells permanently damaged by human diseases. Presently, large scale application for clinical purposes of MSCs is limited because they quickly lose their capacity to replicate and transform when stored under laboratory conditions. Therefore, it is critical for the advancement of Regenerative Medicine to develop techniques whereby MSCs retain their ability to grow and differentate into specialized cells when stored in the lab. Telomeres are capped structures at the ends of chromosomes that are critical to maintaining a chromosome's stability and integrity when cells replicate. Cellular aging is related to the fraying of telomeres when cells replicate. Zalzman et al. (2010) have shown in early mouse stem cells (ES) that a new protein, Zscan4, is able to restore frayed and damaged telomeres, thus allowing cells to replicate and live indefinitely. When Zscan4 is disable, cells lose their ability to replicate indefinitely, and when activated, ES cells become immortal again even in the absence of the protein Telomerase, known to be critical for telomere maintenance. Our hypothesis is that human ZSCAN4 activation in adult MSCs will lead to the restoration of telomeres, thereby allowing both stable, long-term storage under lab conditions and their use in Regenerative Medicine. In this project we will study how human ZCAN4 may function to allow MSCs to survive, replicate and maintain their ability to convert into specialized human cells. Specifically, we will use MSCs acquired from tonsil and bone marrow tissue; these cells are straightforward to obtain and grow well under lab conditions. The goals of this study are to (a) determine how ZSCAN4 affects MSCs capacity to replicate, maintain chromosome stability, and convert into specialized cells when stored under laboratory conditions (b) evaluate if ZSCAN4-activated MSCs are safe in a mouse model and can integrate within normal tissues without forming tumors (c) create a system that allows us to measure how effectively a chemical family, the retinoids, can activate ZSCAN4 in MSCs. Successful completion of this project will allow stable, long term storage of MSCs, while preserving their inherent ability to replicate and transform into specialized cells when stored under laboratory conditions. This will dramatically increase the availability and opportunity for the use of MSCs for Regenerative Medicine.



2013 Exploratoy Grant Awards:

(10)

Jeff W. M. Bulte, Ph.D.
Johns Hopkins University, (JHU)
In Collaboration w/ Cosmeticsurg &
University of Maryland, School of Medicine
Awarded Budged: $226,223.00
Disease Target: Cancer

19F HOT SPOT MRI OF HUMAN ADIPOSE-DERIVED STEM AND PROGENITOR CELLS FOR BREAST RECONSTRUCTION

This proposal seeks to do a first-in-man clinical study on using fluorine-based magnetic resonance imaging (MRI) tracking of stem cells. The initial engraftment and movements of transplanted cells can be imaged with MRI using iron oxide instead of fluorine labeling, but this is often difficult to do after surgical procedures that cause bleeding and tissue injury. Also, the iron oxide labels are not available anymore for clinical use. It is of critical importance if we could determine if the stem cells stay alive after transplantation. In this study, we hypothesize that we can use fluorine MRI as an indirect way to determine and quantify in vivo stem cell survival, while allowing monitoring of their migratory behavior and assisting with guiding needle position for follow-up biopsies. To this end, the Cellular Imaging Section at JHU has partnered with a private practice in Maryland, Cosmeticsurg, where routinely transplantation with the patient's own fat is performed for reconstructive surgery of breast cancer patients with radiation-induced damage of tissue. Purified fat tissue containing stem cells will be labeled with a fluorinated MRI cell label that has been approved by the FDA for its initial use in other cell tracking studies. In this pilot study, 6 patients will undergo liposuction and then receive their own fat with labeled stem cells. The patients will be repeatedly scanned over a period of 1 month at Hopkins. By performing fluorine MRI and counting the total fluorine signal, we hypothesize that we can determine the total cell survival. We expect that a clinically successful outcome (maintenance of breast volume and shape) will be associated with the amount of cell survival. Analysis of tissue biopsies will tell us which type of stem cells survive for at least 4 weeks. The outcome of this study may pave the way for using fluorine MRI cell tracking as a new tool for stem cell therapy in a variety of clinical applications.

Kimberly M. Christian, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $230,000.00
Disease Target: Epilepsy

USING HUMAN NEURONS FOR FUNCTIONAL REORGANIZATION IN A MODEL OF TEMPORAL LOBE EPILEPSY

The recent discovery that adult human cells to be reprogrammed into stem cells has created a revolutionary shift in how we may be able to treat human disease. One of the most exciting avenues that this technology enables is the potential to generate patient-specific neurons for targeted transplantation to repair specific regions of pathology or damage in the adult brain. Many neurological diseases have widespread pathology and so it is very challenging at this stage to envision a straightforward means of exploiting this technology to replace or compensate for damaged or dying neurons that are so dispersed. However, there are several neurological diseases and disorders that have a fairly well-defined region of pathology. Among these, temporal lobe epilepsy (TLE) is a potentially devastating disorder with a specific locus of damage from which epileptiform activity and seizures are generated. TLE presents an optimal opportunity to explore whether transplantation of human neurons or neural precursor cells may be effective to repair dysfunctional brain activity. Specifically, TLE is thought to arise from an imbalance in excitatory and inhibitory signaling due to the selective loss of inhibitory neurons. Restoration of this population through transplantation of healthy cells is an exciting possibility for novel treatment. But the first step toward exploring the utility of this strategy to treat patients is to establish a viable nonhuman animal model in which we can determine the potential of human neurons to integrate into existing brain circuitry and reverse the pathological imbalance in signaling. Rodent models of TLE have been well-established and share many features of human pathology. In this exploratory research project, we propose to test whether transplantation of human neural progenitors are effective in ameliorating the epileptogenic activity in the temporal lobe of rodents with TLE. In addition, we propose to develop a novel platform to assess the viability of transplanted human neurons in rodent models of disease by recording the electrical activity of these cells in living animals. By genetically integrating a light-activated channel into the neural progenitors, we will be able to track these cells in the intact brain and use implanted electrodes and optical fibers to identify these human neurons during electrophysiological recordings and determine whether they have been functionally integrated into the local circuitry. This optogenetic technology has been revolutionary in determining the functional role of specific neuronal populations in nonhuman animals and we propose to adapt this technique to establish a new method of evaluating the functional capacity of human neurons derived from induced stem cells. The successful completion of the proposed experiments will provide critical new data on the utility of animal models to develop translational platforms for novel human stem cell-based therapeutic strategies.

Hugo Guerrero-Cazares, M.D., Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $230,000.00
Disease Target: Multiple

CONTROLLING THE MIGRATION OF HUMAN-DERIVED FETAL NEURAL STEM CELLS VIA SLIT PROTEINS IN A DEMYELINATION ANIMAL MODEL

Brain injuries and diseases such as Multiple Sclerosis, Parkinson's, Huntington's, and Alzheimer's would largely benefit from successful therapies focused on the implantation of fetal and embryonic neural stem cells. These neural stem cells would provide a regenerative source of neurons in damaged brain regions. However, we are still missing a lot of information regarding these cells behavior upon implantation. One key feature in this process is the ability of these cells to migrate within the brain and target damaged areas. Here we propose to study the effects of slits proteins on the migration of human fetal neural stem cells. Slits proteins have the property of directing the migration of neural stem cells in rodents but their effects on human cells has not being tested. Here, we will evaluate this effect on human neural stem cells in vitro and in vivo using healthy and diseased animal models. We anticipate that through understanding the mechanisms that regulate the migration of human neural stem cells, we will be able to target these cells to damaged regions of the brain. Our laboratory has previously described the presence of migratory neural stem cells in the human brain. We have also been successful at extracting and maintaining these cells in culture. In preliminary studies we have observed that slits are able to affect the migration of neural stem cells. Nevertheless, more evaluation is necessary to fully understand the intracellular mechanisms affected upon slits stimulation and their potential therapeutic role in an animal model of a neurodegenerative disease. Given the presence of these migrating NSCs, understanding the mechanisms that guide and direct their migration can potentially be harnessed for regenerative therapy. We aim to evaluate the role that Slit-Robo plays in the migration of human-derived fetal neural stem cells. It is our hypothesis, that Slit proteins will have an effect directing the migration of these cells. Specifically, we will evaluate what protein in the neural stem cells is used as the receptor for slits. Then we will test what intracellular changes occur in neural stem cells upon slits stimulation. Finally, using animal models we will evaluate how this system affects the migration of human neural stem cells in healthy and diseased brain. For this last aim we will utilize a demyelination animal model that recapitulates some of the features present in multiple sclerosis. Through these short-term aims, we hope to accomplish our long term goal of manipulating systems which regulate migration and targeting neural stem cells toward degenerated regions of the brain.

Xiaofeng Jia, M.D., Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $230,000.00
Disease Target: Nerve Injury

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.
The research proposed in this application aims to develop and improve a unique cell-based therapeutic approach with modern genetic engineering techniques and electrical stimulation. This may improve nerve regeneration, and therefore, addresses one of the most challenging aspects for repair of traumatic nerve injuries. 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 proposed 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 project will advance the knowledge of stem cell biology and accelerate the use of stem cell therapies for patient use. Our group is uniquely suited for this research due to our previous knowledge and technical expertise. Both qualities are necessary for successfully completion of the study. Our proposed research will combine multiple aspects of biomedical research to solve critical problems of patient care with peripheral nerve injury. Our technology and protocols are highly translatable to the clinical environment and will provide a significant near-term and long-term benefit to individuals with peripheral nerve injuries. With the PI's clinical background and the human products we use, the unique therapies proposed will be easily translated to clinical use if our hypothesis is proven. Thus, success in this project would greatly enhance the surgical repair of nerve injuries, and offer better functional outcomes. It will also help in developing optimal treatment protocols for orthopedic surgeons specializing in nerve repair.

HanSeok Ko, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $230,000.00
Disease Target: Alpha-Synucleinopathies, PD & LBD

CHARACTERIZATION OF GLUCOCEREBROSIDASE DEFICIENCY PATHWAY IN PARKINSON'S DISEASE

One of the features of Parkinson's disease due to glucocerebrosidase (GBA) mutations results in GBA deficiency and subsequent alpha-synuclein accumulation and neurodegeneration, suggesting that compromised GBA cascade contributes to the pathogenesis of Parkinson's disease (PD) and Lewy Body Dementia (LBD). The molecular mechanisms accounting for GBA deficiency in PD remain elusive. In preliminary studies, we discovered that GIP1, which may function as an ubiquitin E3 ligase for GBA. Preliminary studies indicate that GIP1 interacts with and ubiquitinates GBA. This post-translational modification leads to decreased GBA protein levels and activity and subsequent alpha-synuclein accumulation, suggesting that GIP1 mediated GBA deficiency may contribute to neurodegeneration in PD and LBD. However, all of this work has been done in non-human mammalian systems. In this grant we propose to generate and characterize viral free non-integrating inducible pluripotent stem (iPS) cells from patients with and without GBA mutations and to create conditional GIP1 knockout and control iPS cells to study and characterize the GBA deficiency and the role of GIP1 mediated GBA deficiency in the pathogenesis of PD and LBD. We also propose to perform coupled transcriptome and proteome analysis in DA neurons derived from PD and GBA PD iPS cells to discover biochemical and /or molecular markers that could ultimately be used as biomarkers to monitor the progression of PD and LBD due to GBA deficiency. Ultimately, this project will determine the full implications of the GIP1-GBA-alpha-synulcein neurodegenerative pathway in human DA neurons and identify new targets for therapeutic interventions in PD and LBD by discovering novel approaches to maintaining GBA in a catalytically active and neuroprotective state, and develop human iPS cell models to test new therapies.

Eugene Koh, M.D., Ph.D.
University of Maryland, Baltimore (UMB)
In Collaboration with Pearl Biosciences, LLC
Awarded Budged: $200,000.00
Disease Target: Intervertebral Disc Degeneration

IDENTIFICATION OF SMALL MOLECULES TO DIRECT MESENCHYMAL STEMS CELLS DIFFERENTIATION INTO INTERVERTEBRAL DISC CHONDROCYTES

Degeneration of the intervertebral disc is the leading cause of spinal pathology leading to neck and back pain, at an estimated socioeconomic cost of 30-60 billion dollars per year in the United States. Recent animal studies suggest that regenerating disc cellularity by transplantation of stem cell derived cartilage cells termed chondrocytes may offer a promising alternative to currently available treatments. Adult mesenchymal stem cells (MSCs) are especially good candidate as a source of cells for transplant because they are readily available from bone marrow and can be readily differentiated into chondrocytes. However, the conditions within intervertebral disc are especially harsh, including high mechanical loads as well as low nutrient and low oxygen conditions. In order to survive under these hostile conditions, MSCs will have to be differentiated into specialized intervertebral disc cells termed Nucleus Pulposus (NP) chondrocytes. However, current methods of MSC differentiation lead to the formation of articular instead of NP cartilage. Recent studies demonstrate that extracelluar factors derived from pig notochord cells can specifically differentiate MSCs into NP chondrocytes. However, co-culture with animal cells is not an acceptable method for generation of cells for human transplants. Therefore, there is an urgent need to identify compounds able to direct MSC differentiation specifically into NP chondrocytes. The goal of our proposal is to identify compounds able to direct MSC differentiation specifically into NP chondrocytes as opposed to articular cartilage. To accomplish this goal, we will develop and conduct a high-throughput screen (HTS) of a small molecule chemical library. To facilitate the high-throughput readout for the screen we will take advantage of transcriptional differences between NP and articular cartilage cells. We have already developed a system with a fluorescent reporter readout intended to translate these quantitative differences into a binary HTS readout. This system will allow us a fast and cost effective way to assess the ability of small molecules to direct NP chondrocyte differentiation. Our proposal will result in identification of conditions able to specifically facilitate NP chondrocyte differentiation of MSCs, thus bringing the goal of clinical use of stem cells against intervertebral disc degeneration closer to reality. Additionally, we expect that the identified compounds will be used as tools, to better understand cellular factors and pathways regulating stem cell differentiation into various chondrocyte lineages.

Seulki Lee, Ph.D.
Johns Hopkins University, (JHU)
In Collaboration with Hugo W. Moser Research Institute at Kennedy Krieger (KKI)
Awarded Budged: $230,000.00
Disease Target: Cardiac Regeneration

DESIGN OF HIGHLY FLUORINATED STEM CELLS FOR 19F MR IMAGING IN CARDIAC REPAIR

Transplanted stem cells are anticipated to provide tremendous therapeutic effects in patients; however, certain risks exist by stem cell misbehavior. Therefore, it is vital to validate the therapeutic efficacy and safety of stem cells, and one of the best tools for validation is real-time monitoring of transplanted stem cells in the patient's body. The advances in molecular imaging have significantly contributed to the real-time imaging of transplanted stem cells; however its clinical translation has continued to prove daunting. Recently, one class of magnetic resonance imaging (MRI), 19F MRI, has generated great interest as a "hot spot imaging" technique in the field of molecular imaging and cell therapy communities. The 19F atom is particularly suitable for cell-tracking because of its negligible background in the body; however, the low detection limit of 19F-labeled cells hampers immediate clinical translation of 19F MRI. This can be overcome by preparing highly fluorinated stem cells without altering biological function. In this project, we propose the development of a novel method of preparing highly fluorinated stem cells for 19F MRI applications based on our extensive research experience in both molecular imaging and nanomedicine. Based on our knowledge and experiences related to the design of sophisticated imaging agents, we will develop an innovative nanoformulation for stem cell 19F-labeling (19F-Nano). For the first time and as a model system, human mesenchymal stem cells (hMSCs) will be fluorinated by 19F-Nano and their behaviors in the infarcted myocardium will be noninvasively monitored in a rat myocardial infarction model using 1F/1H MRI. In particular, the overall effect of fluorination on hMSC biological function such as viability and differentiation will be carefully examined to elucidate the safety of using fluorinated stem cells in the clinic. The interdisciplinary approach taken by this research project, involving molecular imaging, regenerative medicine, nanomedicine and stem cell biology, will provide extensive new ideas for future stem cell 19F MRI studies. In addition, success of this project will attract researchers from different fields and promote unique and innovative ideas to ultimately contribute to stem cell-based regenerative medicine and its clinical applications.

Pablo Sanchez, M.D.
University of Maryland, (UMB)
In Collaboration w/ The Living Legacy Foundation & XVIVO Perfusion
Awarded Budged: $200,696.00
Disease Target: Lung Transplantation

BONE MARROW DERIVED STEM CELLS TO IMPROVE DONOR LUNG QUALITY AND TRANSPLANT OUTCOMES.

Two current problems affecting outcomes in lung transplantation are the lack of suitable donors and primary graft dysfunction. Primary graft dysfunction (PGD) is an acute lung injury that occurs in the post transplant period. Clinically and pathologically, the syndrome is similar to acute respiratory distress syndrome (ARDS). PGD is the leading cause of death in the early post transplant period. Treatment options for PGD are widely assorted and vary greatly in effectiveness as most treatments were developed for the treatment of ARDS in the setting of sepsis. Several donor related factors have been associated with PGD, but concerns about the systemic effects of the instituted therapy and the current lung preservation methodology ,cold ischemia, which reduces lung tissue metabolism to around 4%, have limited the development of therapies oriented to improve lung donor quality. Therefore, the possibility of improving organ quality after harvest and before implantation is ideal. It has been demonstrated that Mesenchymal Stem Cells (MSCs) avoid allorecognition, interfere with dendritic cell and T cell function, and generate a local immunosuppressive microenvironment by secreting a number of cytokines. It has also been shown that the immunomodulatory function of human MSCs is enhanced when the cells are exposed to the inflammatory environment. These antiinflammatory and immunosuppressive properties of MSCs make them very attractive as a therapeutic option before, during, and after transplantation. Stem cell therapy may not only modulate the injurious effects of brain death and ischemia reperfusion injury on the donor lung, but also reverse lung failure after lung transplant. This project will use a clinically relevant large animal model of lung transplantation to determine the effects of the administration of MSCs as a therapeutic option to improve donor quality and reduce primary graft failure. In addition the project will involve the use of ex vivo perfused human lungs. We will use lungs that have been rejected for clinical use because are greatly injured to test the effects of MSCs on the improvement of lung donor quality. We believe that this project will have strong implications on the transplant community leading to the development of new therapeutic options that could increase the number of usable donors from the donor pool ,currently 15%, while improving lung transplant outcomes.

Matthew Trudeau, Ph.D.
University of Maryland, Baltimore (UMB)
In Collaboration with Paragon Bioservices, Inc.
Awarded Budged: $230,000.00
Disease Target: Long QT Syndrome

POTASSIUM CHANNELS & HUMAN CARDIOMYOCYTES DERIVED FROM STEM CELLS

We study the properties of a human protein that is found in the heart and helps the heart to maintain the normal heartbeat of once per second. Here we plan to use heart cells, known as cardiomyocytes, for these studies because it is critical to match up the protein with its proper cell type. The cardiomyocytes we plan to use are from "induced pluripotent stem cells" which started out as human, adult skin cells that were turned into stem cells and then cardiomyocytes, meaning that there are not ethical issues with using these cells. In my lab, we study the normal role of a cardiac protein and also the role of mutations in the protein that cause a type of heart disease called a cardiac arrhythmias that can lead to sudden death. In studying the role of these proteins we can better understand what goes wrong in mutant proteins and therefore provide better therapies for individuals with heart disease. In this work we have identified a small fragment of the protein that rescues defective proteins that cause heart disease. Studying these small fragments in cardiomyocytes from stem cells is important because it will tell us if these small fragments might be useful in treating heart disease.

Kathryn Wagner, M.D., Ph.D.
Hugo W. Moser Research Institute at
Kennedy Krieger, Inc.
In Collaboration w/ Johns Hopkins University (JHU)
Awarded Budged: $230,000.00
Disease Target: Muscular Dystrophy

A THREE DIMENSIONAL ENVIRONMENT FOR SKELETAL MUSCLE STEM CELL TRANSPLANTATION

Cell based therapies lag behind the development of other approaches to chronic muscle disease including gene therapy and pharmacological therapies. However, it is commonly believed that cell therapies will provide the eventual cures to chronic inherited and acquired muscle disease such as muscular dystrophy and sarcopenia. Skeletal muscle is composed of syncytial multinucleated myofibers in a fairly simple cellular organization suggesting that stem cell therapy should be quite feasible. Indeed, animal studies have shown engraftment of muscle stem cells and amelioration of disease phenotype. However, previous clinical trials in disease patients have been disappointing. Recognizing that the environment as well as the stem cells needs to be optimized, a new collaborative team composed of Drs. Kathryn Wagner (muscle biologist and neuromuscular clinician), Jennifer Elisseeff (bioengineer) and Gabsang Lee (stem cell biologist), proposes now to develop a three dimensional environment for muscle stem cell transplantation. The Specific Aims of the proposal are to 1) optimize an injectable, biosynthetic scaffold for skeletal muscle stem cell transplantation, 2) embed novel myostatin inhibitors obtained by a collaboration with Pfizer in the biosynthetic scaffold to stimulate muscle stem cells to proliferate and differentiate into myofibers and 3) isolate and derive various human muscle stem cells including satellite cells, pericytes and skeletal muscle cells derived from hiPSCs from human volunteers. The human muscle stem cells will then be seeded on optimized scaffold with embedded myostatin blocker and transplantation efficiency will be assessed in immunodeficient mice. These experiments will provide important knowledge on requirements of biosynthetic scaffolds, properties of various human muscle stem cells and the growth conditions needed to facilitate their transplantation and engraftment to achieve effective skeletal muscle regeneration.

2013 Post Doctoral Fellowship Grant Awards:

(10)

Akshata Almad, Ph.D.
Johns Hopkins University, JHU
Awarded Budged: $110,000.00
Disease Target: Amyotrophic Lateral Sclerosis (ALS)

HUMAN IPSC CELL-DERIVED ASTROCYTES TO STUDY ALS AND ASTROCYTE/ CONNEXINS AS A THERAPEUTIC TARGET

Gap junctions and hemichannels (made of connexin subunits) conduct crucial functions in the central nervous system such as ionic homeostasis, regulation of the excitatory neurotransmitter glutamate, providing metabolic support to neurons, and regulation of neurovasculature and synaptic events. Under pathological conditions such as Alzheimer's disease and spinal cord injury, connexins undergo dysregulation and these vital functions are compromised. ALS is a primary motor neuron disease; however several studies have illustrated the contribution of glial cells such as astrocytes to the disease. In a mouse model of ALS (SOD1G93A mutation), we observe significant increase in protein expression of the astrocyte connexin: connexin 43 (Cx43) at endstage of the disease compared to age-matched control mice. In addition, when functional properties of astrocyte connexins from SOD-1G93A mice were examined in vitro, they were functionally aberrant in comparison to the wild-type astrocytes. We propose to evaluate if astrocytes derived from human induced pluripotent stem cells (iPS) in ALS patients differ in connexin properties compared to control patient samples. We will examine biochemical changes (RNA and protein expression) of connexins and functional changes (dye spreading and calcium waves) between astrocytes from control and ALS patients. We will also determine if co-culturing iPS derived astrocytes from ALS patients with wild type motor neurons mediate neurotoxicity compared to astrocytes from control patients. Finally address if targeting astrocyte connexins can be therapeutic (with specific blockers and siRNA's) and confer protection to neurons cultured with (iPS derived) astrocytes from ALS patients.

Peter Andersen, Ph. D.
Johns Hopkins University, (JHU)
Awarded Budged: $110,000.00
Disease Target: Congenital Heart Defect, Myocardial Infarction

IDENTIFICATION OF CHAMBER-SPECIFIC CARDIAC PROGENITOR POPULATIONS

The mammalian heart is composed of distinct muscle and non-muscle cell lineages: atrial- and ventricular cardiomyocytes, conduction system cells, smooth muscle cells, endothelial cells, endocardial cells and connective tissue. During cardiogenesis, the differentiation of these multiple heart lineages is under tight spatial and temporal control, resulting in the coordinated formation of the four-chambered heart. The four chambers of the adult mammalian heart are specialized in order to handle differing physiological conditions, such as varying degrees of pressure and volume, throughout the adult life. Thus, each chamber displays unique functional, structural, metabolic, and electrophysiological characteristics, reflected by the distinctive gene expression and electrophysical properties. Yet, it is still unclear whether each chamber is derived from pre-specified distinct chamber-specific cardiac progenitors. By a candidate approach based on expression patterns in mice, we have identified surface proteins that define two distinct cardiac progenitor populations, which can be derived from mouse Embryonic Stem Cells (mESCs). By comparing expression patters of these two mESC-derived progenitor populations to the developing right and left ventricle of the embryonic heart, we found a set of genes linking each progenitor population to the left and right ventricle, respectively. These findings suggest, that the heart is derived from at least two distinct chamber-specific pools of cardiac progenitors. Next step will be to confirm these findings in human Embryonic Stem Cells/Induced pluripotent Cells and to confirm the chamber-specificity by tracing these ESC-derived progenitors in the developing heart.

Jing Cai, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $110,000.00
Disease Target: Colorectal Cancer

HIPPO SIGNALING IN INTESTINAL STEM CELL HOMEOSTASIS & CARCINOGENESIS

Intestinal stem cells (ISCs) are required for the normal turnover of the gastrointestinal tracts. Their proliferation must be tightly controlled to maintain tissue homeostasis in response to injuries and excessive proliferation of ISCs may lead to colon cancer. Understanding the molecular mechanisms that regulate ISC self-renewal would therefore facilitate the development of therapies in these human diseases. In my preliminary studies using mouse genetic models, I found that YAP, the nuclear effector of the Hippo pathway, is required for regeneration after intestinal damage and that hyperactivation of YAP leads to intestinal tumorigenesis. Since YAP is highly expressed in normal and cancer stem cells, I hypothesize that YAP is specifically required in the ISCs to promote intestinal regeneration and that YAP is required for the tumorigenecity of colorectal cancer cells. I will test these two hypotheses using mouse genetic models and human colorectal cancer cells, respectively. Given its potential role in ISC homeostasis and carcinogenesis, YAP may represent an excellent target for regenerative medicine and cancer therapies.

Fabien Delaspre, Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $110,000.00
Disease Target: Diabetes

MATURATION OF HUMAN EMBRYONIC STEM CELLS-DERIVED PANCREATIC PROGENITORS INTO INSULIN PRODUCING CELLS

Type1 diabetes (T1D) is an autoimmune disease leading to the destruction of the insulin producing β-cells, the majority of the endocrine pancreas. Insulin is required for regulating blood glucose levels. All patients with T1D and a third of the patients with type 2 diabetes require insulin treatment to prevent hyperglycemia. One approach to recovering glucose homeostasis would be to utilize Stem cell-based strategies, consisting of generating β-cells in vitro and transplanting them into the patient. Several protocols to differentiate β-cells from human embryonic stem cells (hESC) have been proposed, but none succeed in creating mature β-cells. These stepwise protocols recapitulate the key signalling pathways that regulate the formation of the β-cells during embryonic development: endoderm formation, pancreatic specification and endocrine differentiation. Compounds have been identified for inducing endoderm and pancreatic differentiation; yet, none have been described for the maturation of the β-cells in vitro. Our lab uses differentiation of ductal pancreatic progenitors to endocrine cells of the secondary islets in larval zebrafish as a model of β-cell neogenesis. Using this system an unbiased chemical screen was carried out to identify compounds that induce differentiation of pancreatic progenitors to endocrine cells in larval zebrafish. As a result, six FDA-approved compounds were inducing precocious differentiation of secondary islets in larval zebrafish. Recently we started to screen a larger bank of chemicals on the same model and predict the identification of even more candidate compounds. Using available published protocols, we propose to differentiate hESC into insulin-producing cells through endoderm and pancreatic stages, and test the effect of our identified compounds on the maturation of hESC-derived pancreatic progenitors into functional β-cells. The objectives of this protocol would be to obtain differentiated cells showing a glucose-dependent secretion of insulin in vitro and the capacity to restore blood glucose homeostasis in a diabetes mouse model

Jing Fan, Ph.D.
Johns Hopkins University, JHU
Awarded Budged: $110,000.00
Disease Target: Stroke

PARP-1 & HISTONE1 INTERPLAY & REGULATE STEM CELL DIFFERENTIATION AFTER STROKE

Poly (ADP-ribose) polymerase-1 (PARP-1) interacts with histone H1 in the nucleus, and regulates histone modification, DNA methylation, transcription and chromatin structure under physiological and pathological conditions. The mechanisms of PARP-1 and H1 regulation may include relocating H1 from nucleosomes by PARylating it or excluding H1 from some promoters by competitive DNA binding. Several recent studies suggest a critical role for PARP-1 in the developmental programming and control of pluripotent state of Embryonic Stem (ES) cells. Some of the molecular mechanisms of PARP-1 in promoting the differentiation of stem cells, including neuronal differentiation, are through regulating the activity of key stem cell transcription factors, such as Sox2 and HES1. However, whether PARP-1 plays a role in the differentiation and survival of neuronal stem cells after stroke, and whether this PARP-1 regulation is H1-dependent, remain unclear. Thus, experiments will be designed to explore how PARP-1 and histone H1 interact to regulate differentiation and maturation of human neuronal cells after NMDA stimuli and oxygen/glucose deprivation (OGD). I will use human inducible pluripotent stem (iPS) cell culture and cortical neuron cultures generated from iPS cells to explore the transcription factors that are involved in neuron differentiation and survival in NMDA-induced neurotoxicity (Parthanatos) and OGD, by overexpression or shRNA knock-down (KD) of PARP-1 and/or H1 family proteins. The goal of this study is to understand the role of PARP-1 and histone H1 regulation of human neuron differentiation and survival, with hope to find pharmaceutical agents that can promote human neurogenesis and/or differentiation and survival of transplanted neuron cells in stroke patients.

Jeffrey Huo, M.D., Ph.D.
Johns Hopkins University, (JHU)
Awarded Budged: $110,000.00
Disease Target: Hematologic Disorders, Acute Myeloid Leukemia (AML)

THE ROLE OF SOMATIC MEMORY IN DETERMINING EFFICIENT HEMATOPOIETIC DIFFERENTIATION OF HIPSC

Despite best current therapy, acute myeloid leukemia (AML) remains incurable for many patients. It is increasingly understood that the pathogenesis of AML involves establishment and maintenance of a cancerous epigenetic state underlying the coordinated deregulation of constellations of genes, ultimately leading to tumorigenesis. Erasing this cancerous epigenetic state is the basis of the use of epigenetic modifying agents as therapy for AML. However, an enormously complex array of DNA methyltransferases, histone modifiers, and other regulatory proteins have roles in the establishment and/or maintenance of a cancerous epigenetic state. The mechanisms by which these epigenetic regulatory proteins can be coordinated to establish or erase a cancerous epigenetic state remains poorly understood. This lack of understanding is a substantial obstacle towards both optimizing the use of existing drugs for modifying epigenetic state, and development of new, more specific epigenetic modification agents. It is hypothesized that long intergenic noncoding RNAs (lincRNAs) may be a mechanism by which epigenetic regulatory proteins can be coordinated to establish or erase a cancerous epigenetic state. In Aim 1 of this proposal, the recent discovery by the Zambidis laboratory of a mechanism mediating complete functional erasure of epigenetic state in CD34+ hematopoietic progenitor cells, will be combined with work conducted by the Baylin laboratory dissecting the molecular mechanisms of cancerous epigenetic state in CD34+ AML cancer stem cells, to form an experimental comparative genomics strategy to search for candidate lincRNAs that can modify cancerous epigenetic state in AML cancer stem cells. In Aim 2, the ability of these candidate lincRNAs to mediate erasure of cancerous epigenetic state and reduce tumorigenicity will be tested. This work will advance understanding of mechanisms of epigenetic reprogramming, and provide new leads for development of epigenetic modifying therapy and new biomarkers for risk stratification in AML, hematologic malignancies, and cancers in general.

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Johns Hopkins University, (JHU)
Awarded Budged: $110,000.00
Disease Target: Traumatic Brain Injury

ENHANCING THERAPEUTIC POTENTIAL OF HUMAN NEURAL STEM CELLS

Traumatic brain injury has remained as one of the most severe and widespread public health problems. A growing body of evidences has suggested exogenous cell transplantation is one promising strategy to promote injured brain tissue regeneration. However, before stem cell therapy can be a viable option for rehabilitation, many issues still need to be resolved, including low viability, lack control of fate, and low integration after transplantation. The objective of this proposal is to engineer a complimentary approach to direct human embryonic stem cell-derived neural stem cells (NSCs) differentiation into functional neural cells through epigenetic manipulation, while improving survival and function of transplanted cells that repopulate the lesion cavity by promoting vasculature network formation, therefore leading to sustained structural and functional recovery of the damaged brain tissue. Our preliminary data have demonstrated the efficacy of a functionalized hydrogel in promoting formation of a complete vasculature network that fills the lesion cavity. The survival and functionality of NSCs can be significantly improved when hydrogels are used as carriers to re-build the permissive niche for brain regeneration after injury. Recent somatic cell reprogramming has advanced the technologies for transient regulation of the transcriptional network to control stem cell fate specification. Non-viral transfection methods are favored due to the concern of viral vectors regaining reproductive capability or tumor formation through insertional mutagenesis. We propose to use an effective and cell-compatible DNA nanoparticle transfection method to introduce specific transcriptional factors for control of stem cell fate specification in situ. By selecting stage-specific transcriptional factors, neurogenin-2, Mash1 or neuroD, we aim to achieve highly specific control over NSC differentiation and neuron maturation in situ. We will use the optimized protocol to demonstrate the benefits of our approach in moderating structural repair of the lesion and the ensuing improvements in mortality and neurological outcome.

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Awarded Budged: $110,000.00
Disease Target: Diabetes

INDUCED PLURIPOTENT STEM CELL DERIVED, IMMUNOISOLATED B-CELL TRANSPLANTATION FOR DIABETES THERAPY

Diabetes is a worldwide health concern characterized by the permanent destruction or loss of function of insulin-secreting β-cells. Induced pluripotent stem (iPS) cell technology is an attractive strategy being used to generate insulin-producing β-cells for diabetes therapy. In previous studies, iPS cells were generated from somatic cells by transducing four transcription factors using retroviral vectors. The major limitation for potential clinical application of these cell lines is that viral transgenes can integrate into the host genome and cause tumors. Many other alternatives have been reported for generation of iPS cells, including using an adenoviral vector, minicircle, transposon or miRNA, but most of these strategies are either not efficient, don't maintain continuous expression or are cell specific. In this project, we aim to use the most efficient, safe and state-of- the-art technologies to generate β-cells for the treatment of diabetes. A non-integrating, Sendai virus vector would be used to generate human and porcine iPS cells. The novel pluripotent stem cell lines generated by this vector would be characterized and selectively differentiated into insulin-producing, pancreatic β-like cells. We would again use very efficient, two cell-permeable small molecule system, that has been reported to direct differentiation of ~ 80% embryonic stem cells into the endodermal lineage to generate these cells. Insulin-positive cells would then be encapsulated in an alginate membrane followed by intraperitoneal injections of these immunoisolated capsules into diabetic mice models. Treatment efficiency of the capsules would be monitored to confirm that hyperglycemia can be lowered in these mice. Similar studies would also be conducted in large animal models like porcine, before using the cells in humans for diabetes treatment. The long-term goal of this project is to create humanized diabetes models, where each component affected by diabetes can be replaced with the iPS cell-derived tissues.

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Awarded Budged: $110,000.00
Disease Target: Stroke

CELL REPLACEMENT FOR STROKE USING HIPSC DERIVED 3D-ORGANIZED CORTICAL NEURONS

Stroke, one of the leading neurologic disorders associated with severe morbidity and high mortality, results in the loss of critical cortical cells, which the brain is unable to replace. Given the role of stem cells in maintaining and replenishing tissues, stem cell transplantation represents a potential intervention for brain repair and restoration of function and thereby treating the root cause of stroke, cellular loss in this devastating disease. Despite the promise of cell-based therapy for regeneration in stroke, obstacles remain to be addressed to understand the cell replacement mechanisms of human stem cells after transplantation. We have recently developed a scalable and defined system for generating cortical neural stem cells from human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC). These cortical neural stem cells can give rise to highly enriched layer-specific subtypes of cortical neurons in a chemically defined medium. We will transplant cortical neural stem cells into the cortex stroked animals. By using two-photon microscopy, we will observe stem cell survival, proliferation, migration, differentiation and functional integration in the pathological environment. The ultimate goal is to find the optimal approach of cell delivery to the ischemic brain, which can then be responsibly translated to novel therapies.

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Awarded Budged: $110,000.00
Disease Target: Sickle Cell Disease

GENETIC CORRECTION OF SICKLE CELL DISEASE HUMAN IPSC CONVERTED TO A MURINE ESC-LIKE STATE

Induced pluripotent stem cell (iPSC) technology offers new opportunities for treating patients affected by genetic disorders such as sickle cell anemia. Effective and safe iPSC-based therapeutic approaches rely on correcting genetic abnormalities via gene targeting and the efficient generation of corrected stem/progenitor cells for autologous transplantation therapies. Although efficient gene targeting by homologous recombination (HR) and robust generation of engraftable adult-type hematopoietic progenitors are two well-established properties of murine pluripotent stem cells (PSC), both have proved to be inefficient in human cells. FGF-dependent human PSC cultures seem to resemble more closely epiblast-derived cells than conventional LIF-dependent mouse embryonic stem cells (ESC) and recent attempts to switch primed-state human PSC towards a mouse-like state failed to generate stable transgene-free lines . Our laboratory has recently published efficient non-viral non-integrating methodologies to reprogram in bulk human CD34+ blood progenitors into high-quality iPSC. Our preliminary data indicate that we can successfully switch and stabilize our FGF-dependent iPSC cultures into FGF-free LIF-based conditions. Our laboratory has already established robust methodologies to generate hematopoietic progenitors from human iPSC and our group has successfully derived and characterized the erythropoietic progeny of human ESC and iPSC lines harboring the homozygous sickle cell disease (SCD) mutation. Here we aim to assess the HR and hematopoietic efficiencies of mouse-like SCD-PSC lines. First, we will establish and characterize LIF-dependent SCD-PSC lines from our pre-existing primed-state diseased lines. We will assess HR efficiencies in converted SCD-PSC lines and correct in situ the SCD mutation by introducing the wild-type beta-globin sequence. We will assess the hematopoietic progeny of converted LIF-dependent lines (corrected and diseased) and compare it with FGF-dependent parental lines. This proposal will provide a roadmap to achieve safe, efficient, transgene-free correction of monogenic mutations in iPSC derived from patients affected by genetic blood disorders such as sickle cell anemia.

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