MARYLAND STEM CELL RESEARCH COMMISSION

2012 ANNUAL REPORT

MSCRF
Maryland Stem Cell Research Fund

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(Appointed by the Governor)
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(Appointed by the Speaker of the House of Delegates)
Research Geneticist, USDA-ARS, Beltsville, Maryland.

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

2012 GRANT AWARDEES

INVESTIGATOR INITIATED AWARDS {9}

Jeff Bulte
Johns Hopkins University- School of Medicine (JHU)
"Co-Encapsulation of Human Mesenchymal Stem Cells and Islet Cells for Treatment of Type 1 Diabetes"
Awarded Budget: $683,864.00

Ted Dawson
Johns Hopkins University- School of Medicine (JHU)
"Generation and Characterization of Isogenic Parkinson's Disease and Control iPS Cells"
Awarded Budget: $690,000.00

Jennifer Elisseeff
Johns Hopkins University- School of Medicine (JHU)
In collaboration with Tissue Banks International, Inc.
"ECM-Based Materials for Stem Cell Differentiation and Bone Repair"
Awarded Budget: $690,000.00

Dawei Gong
University of Maryland, Balitmore (UMB)
"Towards Modeling Pathogenesis and Treatment of Congenital Generalized Lipodystrophy Using Patient-Specific iPSCs"
Awarded Budget: $600,000.00

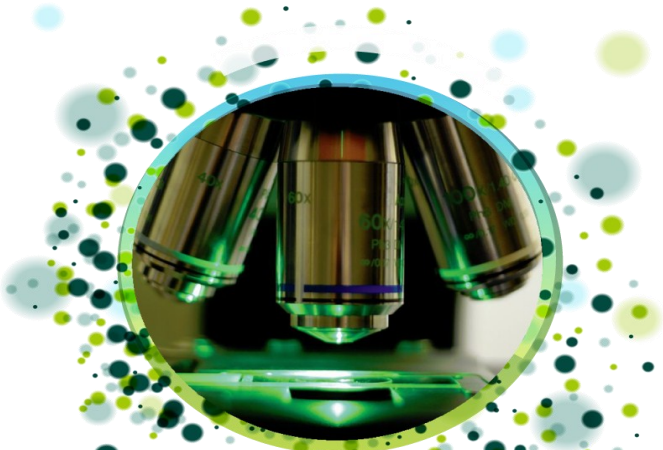
Bartley Griffith
University of Maryland, Baltimore (UMB)
"Improving MSC Engraftment in the Ischemic Heart"
Awarded Budget: $689,958.00

Minoru Ko
Elixirgen, LLC
"Generating Human Induced Pluripotent Stem Cells with Less Cancer-Risk"
Awarded Budget: $690,000.00

Gabsang Lee
Johns Hopkins University- School of Medicine (JHU)
In collaboration with Life Technologies, Inc.
"Derivation of Functional Nociceptive Neurons From hESC and Its Application to Pain-Disorder Human iPSC"
Awarded Budget: $690,000.00

Guo-Li Ming
Johns Hopkins University- School of Medicine (JHU)
"Toward Correction of Neurodevelopmental Defects Of Neurons Derived from Patients with Mental Disorders"
Awarded Budget: $690,000.00

Zack Wang
Johns Hopkins University- School of Medicine (JHU)
"Vascular Progenitor Cell Generation from Human Pluripotent Stem Cells (hPSC) for Peripheral Vascular Diseases"
Awarded Budget: $690,000.00



2012 GRANT AWARDEES

EXPLORATORY AWARDS {17}

Gerald Brandacher
Johns Hopkins University- School of Medicine (JHU)
"Induced Pluripotent Stem Cell (iPS) Derived Schwann Cells to Enhance Functional Recovery Following Nerve Injury and Limb Allotransplantation"
Awarded Budget: $229,905.00

Maria Canto-Soler
Johns Hopkins University- School of Medicine (JHU)
"Development of 3D-Culture Systems of Human iPSC Derived-Retinal Cells for Clinical Applications"
Awarded Budget: $230,000.00

Miroslaw Janowski
Johns Hopkins University- School of Medicine (JHU)
In collaboration with Q Therapeutics, Inc.
"Magnet-Navigated Targeting of Myelin Producing Cells to The Stroke Via Intraventricular Route in a Large Animal Model"
Awarded Budget: $230,000.00

Hongkai Ji
Johns Hopkins School of Public Health (JHSPH)
"Global Prediction of Transcription Factor Binding Sites in Lineage Specific Neuronal Differentiation"
Awarded Budget: $230,000.00

Baktiar Karim
Johns Hopkins University- School of Medicine (JHU)
"CD133: A Master Regulator of Intestinal Differentiation & Stem Cell Regeneration in Crohn's Disease"
Awarded Budget: $229,640.00

Tami Kingsbury
University of Maryland, Baltimore (UMB)
"MicroRNAs and Control of Quiescence and Pluripotency"
Awarded Budget: $230,000.00

Chulan Kwon
Johns Hopkins University- School of Medicine (JHU)
"Membrane Notch Control of Human Cardiovascular Progenitors"
Awarded Budget: $230,000.00

Martin Pomper
Johns Hopkins University- School of Medicine (JHU)
"New ALDH Based Imaging Agents for Stem Cells"
Awarded Budget: $230,000.00

Sivaprakash Ramalingam
Johns Hopkins School of Public Health (JHSPH)
"Functional Correction of hiPSCs with Homozygous Sickle Cell Disease Mutation Using Engineered ZFNs/TALENs"
Awarded Budget: $230,000.00

Antony Rosen
Johns Hopkins University- School of Medicine (JHU)
"Using hESCs to Define Novel Scleroderma Autoantigens in Stem Cells and Vascular Progenitors"
Awarded Budget: $230,000.00

Kara Scheibner
University of Maryland, Baltimore (UMB)
"Regulation of DNA Double Strand Break Repair in Human Hematopoietic Stem Cells by MicroRNAs"
Awarded Budget: $230,000.00

Wenxia Song
University of Maryland College Park (UMCP)
"In Vitro Differentiation of Human Induced Pluripotent Stem Cells Into B-Cells For Modeling Human Diseases"
Awarded Budget: $230,000.00

Joseph Stains
University of Maryland, Baltimore (UMB)
"Using Gap Junctions to Enhance Stem Cell Therapies in Osteoarthritis"
Awarded Budget: $229,412.00

Bhanu Telugu
University of Maryland College Park (UMCP)
"Role of Crucial Oxygen Sensitive Transcription Factor, HIF1A in Stemness and in Disease"
Awarded Budget: $228,259.00

Arun Venkatesan
Johns Hopkins University- School of Medicine (JHU)
"Novel Human iPSC-Based Model Of Axon Degeneration In Multiple Sclerosis"
Awarded Budget: $230,000.00

Mingyao Ying
Hugo W. Moser Research Institute at Kennedy Krieger, Inc. (KKI)
"Highly Efficient Conversion of Human Stem Cells to Dopaminergic Neurons by Proneural Transcription Factor Atoh1"
Awarded Budget: $230,000.00

Steven Zhan
University of Maryland, Baltimore (UMB)
"Modulation of Homing and Engraftment of Hematopoietic Stem Cells by I-BAR Proteins"
Awarded Budget: $230,000.00

2012 GRANT AWARDEES

POST-DOCTORAL FELLOWSHIP {14}

Ola Awad
University of Maryland, Baltimore (UMB)
"Role of Autophagy Dysregulation in the Development of Neurodegeneration Using iPSC Model of Gaucher's Disease"
Awarded Budget: $110,000.00

Amnon Bar-Shir
Johns Hopkins University- School of Medicine (JHU)
"Mri-Based Reporter Genes for Non-Invasive Assessment Of The Fate Of Stem Cell-Seeded Scaffolds"
Awarded Budget: $110,000.00

Su Mi Choi
Johns Hopkins University- School of Medicine (JHU)
In collaboration with Cellomics Technology, LLC
"Patient Specific Stem Cell based In Vitro Model of Liver Cirrhosis"
Awarded Budget: $110,000.00

Christopher Donnelly
Johns Hopkins University- School of Medicine (JHU)
"Development of an Antisense Oligonucleotide Therapeutic Utilizing Stem Cell Derived Patient Astrocytes to Treat ALS and Dementias Caused by C9ORF72 Expanded Hexanucleotide Repeat"
Awarded Budget: $110,000.00

Gabriel Ghiaur
Johns Hopkins University- School of Medicine (JHU)
"Retinoic Acid (RA) Controls Self Renewal and Differentiation of Human Hematopoietic Stem Cells (HSCs)"
Awarded Budget: $110,000.00

Pinar Huri
Johns Hopkins University- School of Medicine (JHU)
"Engineering Clinically-Applicable Vascularized Bone Grafts using Adipose-Derived Stem Cells"
Awarded Budget: $110,000.00

Eunchai Kang
Johns Hopkins University- School of Medicine (JHU)
"Modeling Of Major Mental Disorders Using Human Induced Pluripotent Cells Derived From Patients With A Defined Disc1 Mutation"
Awarded Budget: $110,000.00

Chaekyu Kim
Johns Hopkins University- School of Medicine (JHU)
"Small Molecules to Influence Metabolism in Stem Cells and Tissue Formation in Hydrogels"
Awarded Budget: $110,000.00

Changmei Liu
Johns Hopkins University- School of Medicine (JHU)
"Small RNA Regulation of GSK3 Expression Modulates Human Neural Stem Cells Proliferation and Differentiation"
Awarded Budget: $110,000.00

Georgia Makri
Johns Hopkins University- School of Medicine (JHU)
"Patient-Specific iPSCs for Modeling and Treatment of Rett Syndrome"
Awarded Budget: $110,000.00

Sharyn Rossi
Johns Hopkins University- School of Medicine (JHU)
"Optogenetic Analysis of Stem Cell Integration into Forebrain Circuits Following Traumatic Axonal Injury"
Awarded Budget: $110,000.00

Kit Tsang
Johns Hopkins University- School of Medicine (JHU)
"Epigenetic Regulation of Hematopoietic Differentiation from Human Pluripotent Stem Cells"
Awarded Budget: $110,000.00

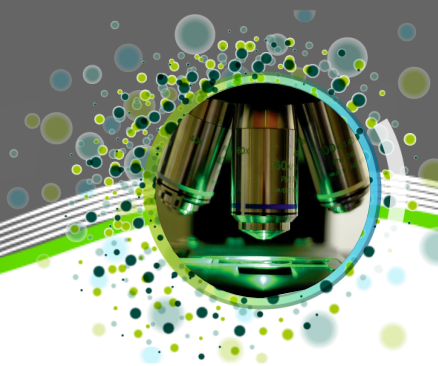
Yi-Lan Weng
Johns Hopkins University- School of Medicine (JHU)
"Effects of DNA Methylation Reprogramming in Axonal Plasticity and Regeneration"
Awarded Budget: $110,000.00

Yaxue Zeng
Johns Hopkins University- School of Medicine (JHU)
"Characterizing the Role of Active DNA Demethylation in Reprogramming of Human Somatic Cell into Stem Cells"
Awarded Budget: $110,000.00

2012 GRANT AWARD ABSTRACTS

INVESTIGATOR INITIATED ABSTRACTS

JEFF BULTE
Johns Hopkins University (JHU)
Awarded Budget: $683,864.00
Disease Target: Diabetes



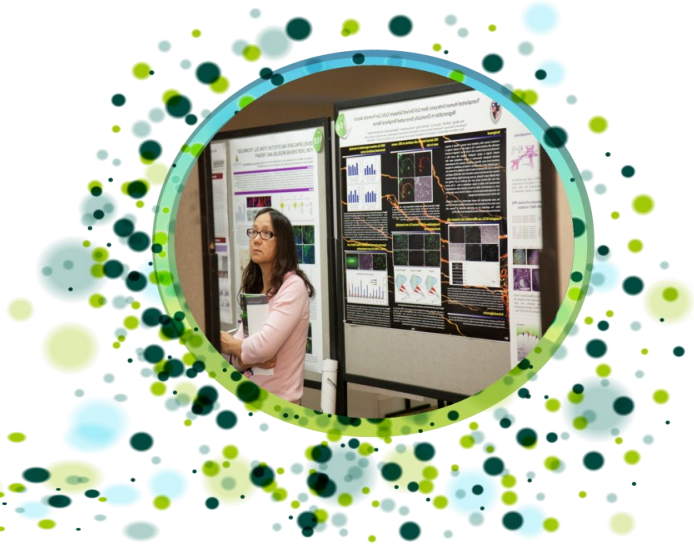
TED DAWSON
Johns Hopkins University (JHU)
Awarded Budget: $690,000.00
Disease Target: Parkinson's Disease

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

For patients with juvenile diabetes, islet transplantation may provide a better and longer lasting cure to restore normal blood sugar levels than can be achieved with an insulin injection pump. Unfortunately, studies from several hospitals have shown that shortly after transplantation most islets stop functioning. This is caused in part by the immunosuppressive medicines that are given to prevent rejection of the transplants. But, recently, special seaweed-derived capsules have been developed that can protect the islets from the outside hostile environment. We have developed special imaging procedures by which we can visualize these seaweed capsules that have islets in them. This is important, as we can then precisely inject them in the blood vessels feeding the liver, we can see if the capsules indeed are arriving at the desired place. And we can determine if the islets stay alive for prolonged periods. To this end, we load the capsule with particles that has fluorine and protamine sulfate in it. This make the capsules appear as bright spots on MRI scans and trap oxygen that keeps the cells alive. Transplantation of these capsules in the belly of diabetic mice has resulted in rescue of these mice with blood sugar levels returning to normal levels. We now want to study a new approach in which human mesenchymal stem cells (MSCs) are co-transplanted with the human islets. Some people have found that these MSCs secrete molecules that can slow down the immune attack against the capsules and can also induce the body to make new blood vessels which in turn provide more nutrients and oxygen for the human islets. We have done experiments in both the petri dish and in live animals that show proof-of-principle that this co-encapsulation approach may work. We would like to study these co-encapsulated islets in diabetic mice, and use a light-emitting imaging technique copied from the firefly to see if the islets are surviving better in the presence of these stem cells. Based on the mice studies, we will then choose the optimal cell conditions for further studies in diabetic pigs. The materials we are using are already used in the clinic for other studies. We believe our overall studies may solve some of the current problems that now in exist in islet transplantation for treatment of juvenile diabetes, and hopefully someday lead to a cure.

Generation & Characterization of Isogenic Parkinson's Disease & Control iPS Cells

One of the unifying features of Parkinson's disease is mitochondrial dysfunction. The molecular mechanism's accounting for the mitochondrial abnormalities in Parkinson's disease are not known. In this grant we propose to generate and characterize viral free non-integrating inducible pluripotent stem (iPS) cells from patients with parkin mutations and to create conditional parkin knockout and control isogenic iPS cells to study and characterize the role of parkin inactivation in the pathogenesis of PD. This project will also explore the role of PARIS, a novel protein that we identified that appears to play a prominent role in Parkinson's disease by causing mitochondrial dysfunction. We will use state-of-the-art technology to identify genes that are regulated by PARIS and determine whether they play a role in Parkinson's disease. Since Parkinson's disease is due to the selective loss of dopamine neurons, studies will be conducted to uncover critical determinants that regulate expression of PARIS, PGC-1£\ and NRF-1 in human dopamine neurons. This will be followed by the evaluation of the role of PARIS, PGC-1fÑ and their target genes and their relationship to mitochondrial dysfunction in PD. Ultimately, this project will determine the full implications of the parkin-PARIS-PGC-1£\ neurodegenerative pathway in PD. Through these studies we will iden-tify new targets for therapeutic intervention in Parkinson's disease by discovering novel approaches to maintain mitochondrial function and the development of human inducible pluripotent stem cell models to test new therapies.



JENNIFER ELISSEEFF
Johns Hopkins University- (JHU)
In collaboration w/ Tissue Banks International
Awarded Budget: $690,000.00
Disease Target: Multiple

DAWEI GONG
University of Maryland, Baltimore (UMB)
Awarded Budget: $600,000.00
Disease Target: Lipodystrophy

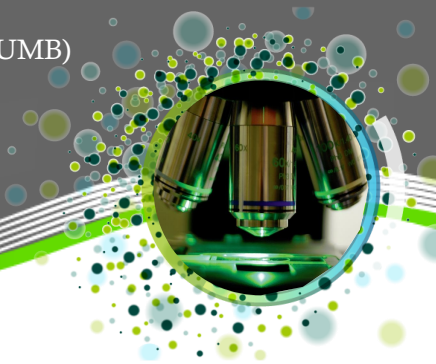
ECM-Based Materials for Stem Cell Differentiation and Bone Repair

Regenerative medicine is a field that aims to address the challenges of tissue loss due to trauma, disease or congenital abnormalities. Tissue engineering is one approach for regenerating new tissues that utilizes as a biomaterial scaffold to support and stimulate tissue development from cells in surrounding host tissue or delivered exogenous. The overall objective of the proposed research is to create novel biomaterial implants that combine the extracellular matrix from tissues with synthetic materials. The extracellular matrix (ECM) from tissues contain much of the biochemical and architectural complexity a tissue that play an important role in directing stem cell fate and to promote tissue repair clinically. These materials are currently used in the tissue banking industry but often lack practicality from the clinical application perspective since the shape, design and mode of implantation are often uncontrolled. We propose to design composite biomaterials that incorporate native ECM within the context of synthetic nanofiber and hydrogel materials. The ability of the new scaffolds to direct stem cell differentiation will be evaluated and one group will ultimately be tested for their ability to regenerate bone tissue in a preclinical model. While the materials design strategy will be employed with connective tissue, the technique can be generalized to include matrix from many other tissues including heart, brain, and liver.

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

Genes code for proteins, the molecules that carry out most of the work in a living organism. When a gene is mutated so that its protein product can no longer carry out its normal function, a disorder can result. There are more than 6,000 known single-gene (also called monogenic) disorders, which occur in about 1 out of every 200 births in general human population. Some well-known disease examples are cystic fibrosis, sickle cell anemia, Marfan syndrome, Huntington's disease, and hereditary hemochromatosis. Monogenic disorders can run in families or sporadically. Apparently healthy parents may give birth to a child with monogenic disease. Currently, there is no cure for monogenic disease and treatment usually aims for symptom alleviation. Many monogenic diseases are often fetal and devastating, imposing an extreme metal, physical and financial burden to patients, their families and society. Thus effective treatment has been sought to treat monogenic disorders. Recent advances in stem cell research and DNA recombination technology have made it possible to repair genetic defect for treatment of monogenic diseases. For example, a patient's skin cells can be first turned into induced pluripotent stem cells (iPSCs), followed by repair of the genetic defect by introduction of a good, functional gene. And the resulting mutation-corrected iPSCs can then be amplified and induced to become a desired type of cells, e.g. hepatocytes, nerve cells, blood cells, for cell-based therapy. Although promising, the approach requires thorough and rigorous research before it can be used for the clinic. As for any other science and technology development, good research model will facilitate the iPSC-based therapy to move forward early clinical applications. This application aims to repair the genetic defect of congenital generalized lipodystrophy disease 1 (CGL1) by using the iPSCs technology. CGL1 is a rare, devastating disease due to a mutation and deficiency of AGPAT2 metabolic enzyme, and the patients are totally lack of fat tissue, and have diabetes and fatty liver. In addition, patients are in distress for social life because of the absence of the face and body fat. There is no effective treatment for the disease. We propose to generate iPSCs from CGL1 patient's skin cells and next repair the genetic defect by replenishing functional AGPAT2 in the iPSCs, which are then tested to see if the repaired cells will become fat cells. Since it would take time before the cell-based therapy can be tried in the patients, we will simulate the entire treatment steps in animals. We expect the success of the proposed study will lead to

BARTLEY GRIFFITH
University of Maryland, Baltimore (UMB)
Awarded Budget: $689,958.00
Disease Target: Multiple



MINORU KO
Elixirgen, LLC
Awarded Budget: $690,000.00
Disease Target: Multiple

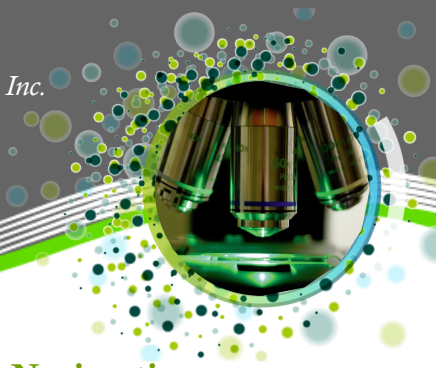
Improving MSC Engraftment in the Ischemic Heart

Cellular therapy is an important component of regenerative medicine for the 21st century. Several different types of stem cells are under evaluation as a component of cellular therapy. Mesenchymal stem cells (MSCs) have become a "standard" of multipotential cell therapy and they are used in very diverse strategies from repairing bone non-unions to improving ischemic hearts to treating death bed grade IV GVHD patients. Despite their wide use over a number of years there are still a number of issues for MSCs that are not well understood. One important issue is optimizing the engraftment of MSCs so that the benefits of these stem cells can be more fully realized. Although readily expanded in culture, MSCs are highly contact inhibited, and do not proliferate appreciably in vivo so their optimal delivery is essential to realizing their full therapeutic potential. Over a decade has gone by since we first put MSCs into the infarcted heart in animal models and it has been 5 yrs since the first human cardiac clinical trials with MSCs were begun. Nevertheless, limited progress has been made towards optimizing the clinical delivery and survival of MSCs in the infarct environment. For example, currently for the treatment of heart attack patients, 100 million or more MSCs are delivered as a therapeutic dose, but only ~ 1% remain a short time later. Our hypothesis is that improving engraftment will lead to improving cellular therapy for the infarcted heart. Improving the engraftment of MSCs is a necessary component for developing better stem cell therapies and evaluating the potential of MSCs to regenerate the damaged myocardium. We will evaluate methods to treat MSCs ex vivo and examine cell survival in stress conditions in vitro. We will further examine these methods in promoting in vivo survival in a rat cardiac ischemia/repurfusion model, using athymic rats to allow delivery of the human MSCs. Further, a large animal pig ischemia/repurfusion model will be tested as a prelude to clinical considerations. As MSC-based therapies are under development for a number of conditions, strategies for improved cellular engraftment in the damaged heart should be important in these other therapies as well. The data from this project should also be useful for researchers using other stem cells too.

Generating Human Induced Pluripotent Stem Cells with Less Cancer-Risk

The current major paradigm in the field of regenerative medicine is to make induced pluripotent stem cells (iPSCs) from patients' fibroblast cells, differentiate them into desired cell types, such as dopaminergic neurons for Parkinson's disease, and transplant them back to the patient. The iPSCs, which can be differentiated into essentially any cell type, can be generated simply by the forced induction of four transcription factors in cell culture. The method has been widely considered as the choice to generate pluripotent stem cells, as it does not include the destruction of human embryos. However, it has been recently shown that the iPSCs generated with the current method are prone to have mutations, that is, alterations in the DNA sequences. This has raised serious concerns about the safety of iPSCs for use in Regenerative Medicine. If the transplanted cells are prone to become cancers, such cell transplantation therapy could cause more harm than good. Therefore, without solving this issue, regenerative medicine may never be realized. We have recently discovered a gene which plays an important role in the maintenance of genome integrity and normal karyotype in mouse embryonic stem cells. We have also demonstrated that the same gene can enhance the efficiency of iPSC formation and increase the quality of iPSCs in the mouse model system. In this grant application, we would like to test whether this gene can also have the same beneficial effects on

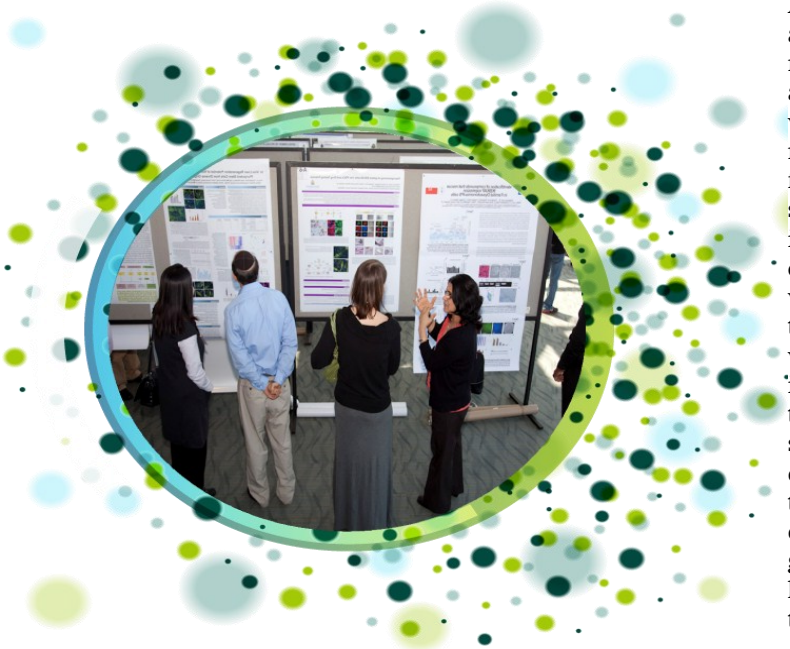
GABSANG LEE
Johns Hopkins University (JHU)
In collaboration with Life Technologies, Inc.
Awarded Budget: $690,000.00
Disease Target: CIPA



GUO-LI MING
Johns Hopkins University (JHU)
Awarded Budget: $690,000.00
Disease Target: Schizophrenia

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

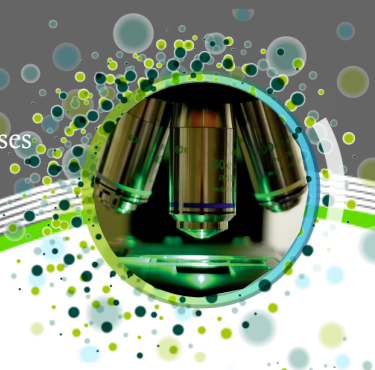
Human pain-sensing neurons are the first cellular component in our body for detecting and mediating exogenous stimuli to protect us from any potential danger. Often this 'alarm' system is not properly functioned and causes undesirable pain-sensation, which ends up unbearable pain. Unfortunately pain disorder is one of the major symptoms that drastically decrease the quality of life. Currently most of the study related with pain is based on rodents that often present irrelevant phenotypes and results than that of humans, which suggests new human system. Understanding human pain sensation has been hardly possible, mainly due to scarcity of patient samples. Recently human pluripotent stem cells are believed to be an alternative for many research purposes including pain study, but harnessing the potential of human pluripotent stem cells into pain-sensing neurons has been challenged. Our group already accumulated significant expertise on peripheral neuron specification with human pluripotent stem cells over 5 years. We



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

Many neuropsychiatric disorders, including schizophrenia, schizoaffective disorder, 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 environmental factors 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. Taking advantage of this technology, we have generated iPSCs from seven 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. Our preliminary data suggest that neurons derived from the iPSC lines with a DISC1 mutation show aberrant structural development, which is likely to affect the functional integrity of these cells. In the current proposal, we aim to fully characterize the developmental trajectory of human neurons with and without this DISC1 mutation by analyzing the structural and functional properties of these neurons over time. We will determine whether the DISC1 mutation impacts the ability of neurons to form functional networks in the brain through transplantation of neural precursors to mice. Finally, we will screen for pharmaceutical approaches to rescue any observed deficits using a collection of bioactive molecules and a collection of anti-psychotic drugs for the treatment of psychiatric disorders. In summary, we will carry out a systematic investigation of how a single risk gene impacts the development of human neurons and to identify potential treatment in reversing those phenotypes.

ZACK WANG
Johns Hopkins University (JHU)
Awarded Budget: $690,000.00
Disease Target: Peripheral Vascular Diseases

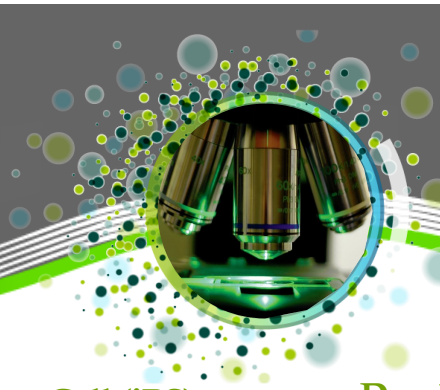


Vascular Progenitor Cell Generation from Human Pluripotent Stem Cells (hPSC) for Peripheral Vascular Diseases

Peripheral vascular diseases (PVD), also called peripheral arterial diseases (PAD), are caused by chronic inadequate blood flow in the lower extremities, resulting in critical limb ischemia and eventually limb amputation. The increasing incidence of PVD has become a growing medical problem. PVD patients are often associated with aging, diabetes, obesity and hypertension, and are unable to revascularize damaged tissues because of a reduced number and functionality of circulating endothelial progenitor cells. Transplantation of vascular cells from other sources offers a potentially noninvasive approach for repairing ischemic tissues in PVD patients. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced-pluripotent stem cells (hiPSCs), are capable of self-renewal (producing more stem cells) and differentiation into various tissue cells, including cardiovascular cells. Therefore, hPSCs represent an important cell source to treat vascular diseases. However, transplantation of undifferentiated hPSCs results in tumor formation. Vascular progenitor cells have potentials to expand in vitro and to give rise to two major building blocks and functional components of blood vessels: endothelial cells and smooth muscle cells. Thus, characterization of hPSC-derived vascular progenitor cells is a crucial step leading to safe and effective cell therapies for vascular diseases. In this project, we will investigate how hPSC-derived vascular cells form blood vessels most effectively following transplantation in a mouse model of PVD, and investigate the factors that promote and stabilize blood vessel formation from hPSC-derived vascular cells. The signals that regulate hPSC differentiation into vascular progenitor cells are largely unknown. We will further characterize a molecular regulator that directs the development of vascular progenitors from hPSCs. Patient-specific hiPSCs can circumvent some of the therapeutic limitations of hESCs, such as ethic concerns and immunogenicity/allograft rejection. Although most of our preliminary studies were based on hESCs, we will compare the vascular cell generation of hESCs with that of hiPSCs in our established system. Our results should have important positive impacts on the robust generation of vascular cells for cell-based therapies of vascular diseases.


EXPLORATORY
ABSTRACTS

GERALD BRANDACHER
Johns Hopkins University (JHU)
Awarded Budget: $229,905.00
Disease Target: Multiple



MARIA CANTO-SOLER
Johns Hopkins University (JHU)
Awarded Budget: $230,000.00
Disease Target: Retinal Degenerative Diseases

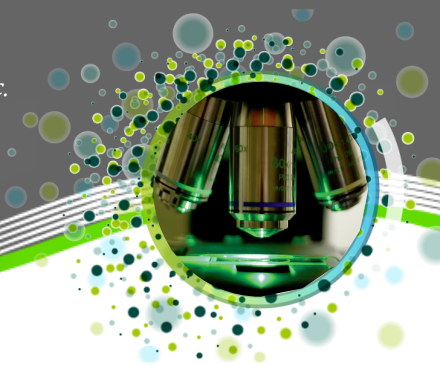
Induced Pluripotent Stem Cell (iPS) Derived Schwann Cells to Enhance Functional Recovery Following Nerve Injury and Limb Allotransplantation

Organ transplants are now common place, today hand, arm, knee and face transplants are becoming a clinical reality allowing people to regain missing limbs and facial tissue, all transplanted from a donor. The investigators of the proposed study have performed a number of hand and arm transplants in the US including the first bilateral hand and the first above elbow transplant with great success. The current limiting issue facing these types of surgery is the slow speed and rates of nerve regeneration. These innovative reconstructive transplant surgeries could be expanded to include full leg and full arm transplants, if this limitation could be overcome. This proposal is to study enhanced functional recovery through the use of patient specific stem cells, with the ultimate goal of broadening the clinical scope of reconstructive transplantation. In the future, these therapies could also have direct clinical application for thousands of nerve injuries incurred every day. Specifically, we propose a novel stem cell-based therapy utilizing induced pluripotent stem cell (iPSC)-derived Schwann Cells to enhance nerve regeneration and improve functional outcomes in reconstructive transplantation. iPSC-derived stem cells and cells overexpressing growth factors will be delivered to an injured nerve in an experimental limb transplantation model in rodents. The impact of this therapy on the functional recovery of nerve regeneration will be monitored and compared through sophisticated experimental techniques including computer assisted video gait analysis. The results of our pre-clinical studies can be directly and immediately applied to ongoing clinical trials of hand, arm and face transplants at Johns Hopkins. If successful, this project will serve as the groundwork for new stem cell based methods to enhance nerve regeneration potentially broadening the clinical utility of extremity transplantation and providing new hope of restoration and functional recovery for hundreds of thousands of people with upper or lower limb loss.

Development of 3D-Culture Systems of Human iPSC Derived-Retinal Cells for Clinical Applications

Retinal degenerative diseases are a group of clinical conditions in which the death of retinal photoreceptor cells leads to vision loss, and some times, total blindness. Despite significant effort directed toward understanding these diseases and the development of therapeutic treatments, there is still no cure for them. The use of stem cells raises great enthusiasm due to their potential clinical applications. In particular, the development of induced pluripotent stem cells (iPSC) a few years ago has open previously unthought-of possibilities for the use of stem cells for disease treatment. Human iPSC are generated from adult cells, as for example skin or blood cells, obtained through a routine clinical biopsy. In the laboratory, these cells are then forced to express a group of specific genes that instruct them to "go back in time" and acquire an "embryonic stem cell-like" status. These cells can then be re-directed to differentiate into any cell type of the adult body and used to study specific diseases and to develop therapeutic treatments. Therefore, the long-term goal of this project is to develop human iPSC based-technologies for the study and treatment of retinal dystrophies. Specifically, we will aim at developing three-dimensional culture systems that would induce hiPSC to differentiate into retinal cells, and in particular photoreceptor cells, with the efficiency and to the extent required for potential clinical applications. The development of this technology will provide an ideal biological system for the study of the mechanisms leading to these diseases and the discovery of drug-based therapeutic treatments. In addition, it will also provide a special context to assess the potential of patient-specific iPSC as a source of autologous cells for retinal transplantation.

MIROSLAW JANOWSKI
Johns Hopkins University (JHU)
In collaboration with Q Therapeutics, Inc.
Awarded Budget: $230,000.00
Disease Target: Strokes



HONGKAI JI
Johns Hopkins School of Public Health
Awarded Budget: $230,000.00
Disease Target: Multiple

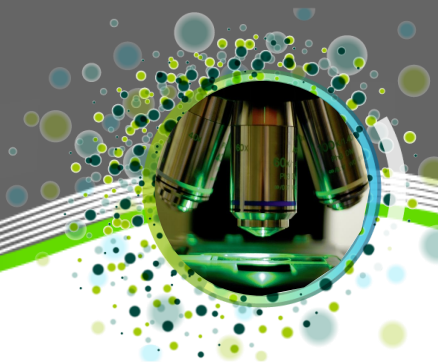
Magnet-Navigated Targeting of Myelin Producing Cells to The Stroke Via Intra-ventricular Route in a Large Animal Model

Cell therapy has a tremendous potential to overcome limitations of current treatment methods of neurological disorders. Stroke is the leading cause of long-term, severe disability, it is the major disease burden to society. Several clinical neurotransplantation trials for stroke are underway worldwide. However, no robust therapeutic effect was yet achieved to make this approach a clinical routine. Moreover, optimal route of cell transplantation was not yet defined. Various cell types with different mechanisms of action were evaluated and there is no consensus on the best candidate. The most frequent routes of cell transplantation include intravascular or intraparenchymal, both are not devoid of limitations. Recent case report of intraventricular delivery of iron oxide labeled cells followed by non-invasive monitoring of cell fate by MRI in a pediatric patient has proven this method safe and feasible. However, after injecting cells to cerebral ventricles their distribution is governed by basic physical forces such as sedimentation and to date there was no method allowing control over that distribution. Thus we investigated in vitro if cells loaded with iron oxide and suspended in fluid can be pulled by magnet. We have shown that low cost neodymium magnet, only two inch in diameter is sufficient to guide cells within a range of 10 cm. This distance is more than from surface of the head to the cerebral ventricles, which are filled with cerebro-spinal fluid, thus we can potentially guide cells within ventricular system toward the part of the brain stricken with stroke reducing the need for long-distance migration of cells within the brain parenchyma. Since navigation of iron loaded cells with the magnet is highly dependent on the brain size, we propose to use large animal to better model conditions of human brain. We selected porcine model of stroke due to its anatomical conditions it provides better translatability of this approach to clinical application. Neurons and mesenchymal stem cells are most frequently employed in clinical stroke trials, but did not bring expected breakthrough. It has been shown that the level of disability and prognosis for recovery is not related to the size of stroke, but is determined by the involvement of white matter cortico-spinal tract. White matter consists of oligodendrocytes forming around of the axons a myelin sheath responsible for the proper conductivity of neuronal impulses. Myelin producing cells were shown to be very sensitive to ischemia, and their injury results with improper function of axons what in turn escalates neurological deficits. We propose to supply infarcted tissue with exogenous myelin producing cells.

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

Stem cells or their derivatives may be used to treat human diseases by replacing damaged cells. The ability to differentiate stem cells and their derivatives into the correct cell types will be crucial for developing future stem cell based therapies. Acquiring such ability requires knowledge of how cells control gene activities during the differentiation. The binding of transcription factors (TFs), a special type of proteins, to cis-regulatory DNA sequence elements is one important mechanism used by cells to modulate gene activities. Humans have approximately 1400 TFs, each may have tens of thousands of DNA binding sites in the genome. The TF-DNA binding is highly context dependent. A comprehensive understanding of gene regulation would require one to map all DNA binding sites of all TFs in all relevant biological conditions, which is difficult to achieve using current technologies. The state-of-the-art technologies, ChIP-seq and ChIP-chip, can only map genome-wide binding sites for one TF in one biological condition at a time. Applying these technologies to analyze many TFs and many biological conditions is costly, labor intensive, and requires high-quality antibodies which are not always available. As a result, it does not provide a feasible solution. The objective of this proposal is to develop a new approach that integrates experimental and computational techniques to simultaneously map genome-wide binding sites of 500+ TFs in many biological conditions. In this approach, investigators use mature technologies to obtain gene expression profiles of biological samples of interest. They then use the gene expression profiles as predictors to predict genome-wide binding sites of 500+ TFs in each sample. The prediction model will be trained using the publicly available gene expression and DNase I hypersensitivity data generated by the ENCODE project, and the information about TF binding motifs. This approach will offer a cost-effective solution to globally map binding sites of many TFs in many biological conditions. We will test and validate this approach using a well-controlled system of neural progenitor cell (NPC) differentiation. NPCs derived from human ESCs and iPSCs will be differentiated into two neuronal lineages. Understanding gene regulation are highly relevant to developing stem cell based therapies for Parkinson's disease and motor neuron disease. We will generate time course gene expression data , predict genome-wide transcription factor binding sites, and identify and experimentally validate TFs that play important roles in the lineage specific neuronal differentiation.

BAKTIAR KARIM
Johns Hopkins University (JHU)
Awarded Budget: $229,640.00
Disease Target: Crohn's Disease



TAMI KINGSBURY
University of Maryland, Baltimore (UMB)
Awarded Budget: $230,000.00
Disease Target: Transplant Relevant Diseases

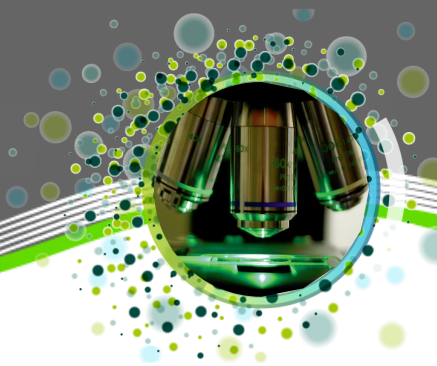
CD133: A Master Regulator of Intestinal Differentiation & Stem Cell Regeneration in Crohn's Disease

Crohn's disease is an inflammatory disease of the intestines. It affects more than 400,000 people in United States. Genetics, diet, bacteria, and autoimmune disease may be the causative agents of the Crohn's disease. Crohn's disease is a chronic condition without cure and is associated with colorectal cancer. Medication and intestinal surgery is the main treatment strategy but it is clearly not curative. We focus on stem cell technology to reverse and repair the intestinal mucosa. Over expression of CD133 (stem cell marker) is a promising strategy for treating Crohn's disease. The proposed studies are design to elucidate the role of stem cells in intestinal regeneration in gastrointestinal diseases such as Crohn's disease. Finally, in this proposal we will provide strategies for intestinal repair in Crohn's disease.

MicroRNAs and Control of Quiescence & Pluripotency

Low levels of cellular reactive oxygen species (ROS) levels are a hallmark of quiescent stem cells and have been implicated in maintaining stem cell integrity and pluripotency. Although stem cells hold great promise for regenerative medicine, their clinical application is limited by our inability to ex vivo expand stem cells without changing their cell properties. MicroRNAs are small RNAs that regulate gene expression post-transcriptionally via modulation of mRNA translation or stability. Several miRs involved in mitochondrial function and ROS generation in differentiated cells and cancer have been reported. In this exploratory proposal, we will identify miRs capable of reducing ROS levels in hematopoietic stem-progenitor cells (HSPCs), the most well-defined population of human stem-progenitor cells available. We predict that enforced expression of miRs targeting mRNAs encoding proteins directly involved in ROS generation or signaling pathways that stimulate ROS production or accumulation will reduce ROS levels and thus become enriched within the ROSlow cell sub-population. HSPCs will be transduced with a miR library and then separated into ROSlow vs. ROShigh cell populations via fluorescent activated cell sorting. Quantification of miR library integrants in the two cell populations will reveal which miRs are enriched in ROSlow population. Candidate ROS-diminishing miRs will be individually transduced into HSPCs to verify their ability to reduce ROS levels. The effect of validated ROS-diminishing miRs on HSPC CD34+ expression and mitochondrial number and function will be tested in HSPCs during a time course of cells in culture. If ROS reduction is sufficient to maintain HSPC-properties, we expect ROS-diminishing miR expressing HSPC cultures to retain a higher percentage of CD34+ cells with low mitochondria function compared to control cells. The functional consequence of ROS-diminishing miRs on hematopoietc progenitor (HPC) cells will be tested by comparing the number of in vitro colony forming cells in miR-expressing vs. control cells. Hematopoiteic stem cell (HSC) function will be investigated using a mouse transplant model and assaying the ability of ROS-diminishing miRs to enhance engraftment and human hematopoiesis. The results of these analyses will determine the ability of ROS to increase HSPCs numbers during time in culture and test the potential of ROS-diminishing miRs to provide a novel approach for ex vivo expansion of these critical cells to generate sufficient HSPCs for patient transplants. ROS-diminishing miRs will also provide valuable tools to dissect the molecular mechanisms regulating ROS levels in HSPCs.

CHULAN KWON
Johns Hopkins University (JHU)
Awarded Budget: $230,000.00
Disease Target: Multiple



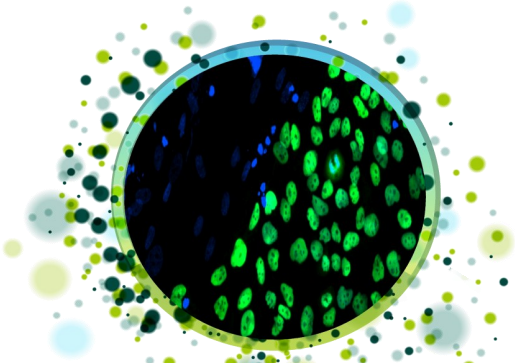
MARTIN POMPER
Johns Hopkins University (JHU)
Awarded Budget: $230,000.00
Disease Target: Cancer

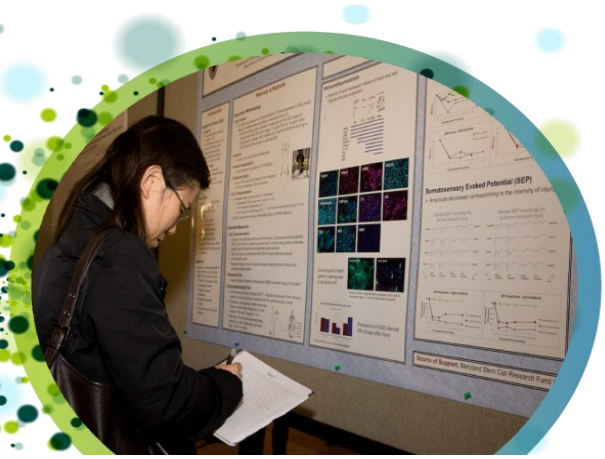
Membrane Notch Control of Human Cardiovascular Progenitors

Understanding the biology of the cells that develop into the heart, called multipotent cardiovascular progenitor cells (CPCs), is key to realizing the promise of future cell-mediated cardiac therapeutics. The proposed research aims to elucidate the ligand/transcription-independent role of a protein called Notch in human CPC self-renewal and differentiation. Our work will provide mechanistic insights into understanding the self-renewal-differentiation processes of multipotent CPCs regulated by Notch, which will facilitate future cell-based cardiac therapeutics and open new avenues of investigation for non-canonical Notch biology.

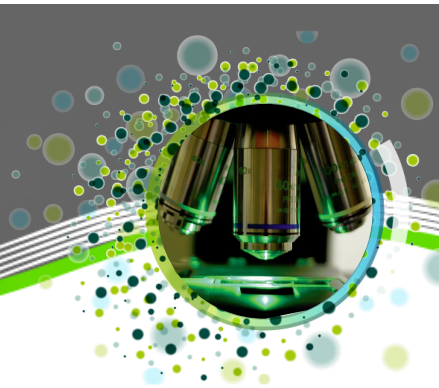
New ALDH Based Imaging Agents for Stem Cells

Although new cancer therapies are continually emerging and are more effective and less toxic then those of prior generations, the vast majority of patients will eventually relapse as the tumors reoccur and metastasize. One reason that may be happening is that there is a subpopulation of cells within the tumor, referred to as cancer stem cells (CSCs) that are not adequately treated by existing chemotherapy. It is that minority of cells that is responsible for tumor reoccurrence and ultimate resistance to chemotherapy. We are purposing a method to image the CSCs so that we can find where these cells are located in the tumor so that they can be better targeted surgically or otherwise. By having imaging agents specific for CSCs, we will be able to perform non-invasive, image-based therapeutic monitoring in patients undergoing cancer chemotherapy or, in the case of optical compounds, we could perform real-time surgical guidance. As a corollary to this work we will be able tag non-malignant stem cells in general, which might be used for future cell-based therapies. To accomplish these goals we intend to synthesize radioactive and fluorescent substrates of the enzyme aldehyde dehydrogenase (ALDH), which is overexpressed in stem cells. We will then test the compounds that we synthesize in cells that either express or do not express ALDH as well as in relevant animal models. At the end of this funding period, we will have one or more compounds that will be ready to undergo additional toxicity testing and other studies to enable human imaging with radionuclide (positron emission tomography and/or single photon emission computed tomography) or optical methods.





SIVAPRAKASH RAMALINGAM
Johns Hopkins School of Public Health
Awarded Budget: $230,000.00
Disease Target: Sickle Cell Disease



ANTONY ROSEN
Johns Hopkins University (JHU)
Awarded Budget: $230,000.00
Disease Target: Multiple

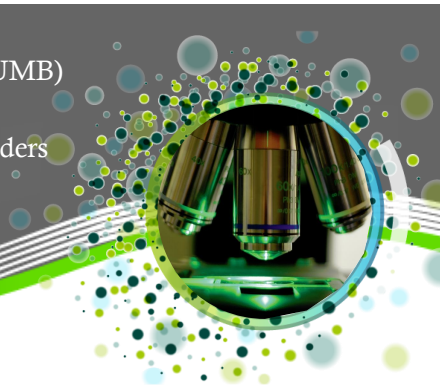
Functional Correction of hiPSCs with Homozygous Sickle Cell Disease Mutation Using Engineered ZFNs/TALENs

A major challenge in the development of Genetic Medicine is to modify the human genome at specific sites in cells. Zinc finger nucleases (ZFNs) are proteins custom-designed to cut at specific DNA sequences. Because the recognition specificities of the ZFNs can be easily manipulated experimentally, they offer a general way to deliver a targeted double-strand break (DSB) to the genome. ZFNs have become powerful tools for enhancing gene targeting – the process of replacing a gene within a genome by homologous recombination (HR) in cells. Site-specific engineering of the human genome so far has been hindered by the low frequency of HR in human cells. In ZFN-mediated gene targeting, this is circumvented by using designed ZFNs to cut at the desired chromosomal locus inside the cells. The DNA break is then patched using the new investigator-provided genetic information and the cells' own repair machinery. The high efficiency of this process combined with the ability to design ZFNs that target almost any DNA sequence makes ZFN technology a powerful research tool for targeted engineering of the genome of human primary and stem cells. The current gene therapy protocols, which are based on gene addition approaches, try to compensate for defective genes by randomly inserting a new working copy into cells. These approaches are beset with complications arising from the random insertion of the therapeutic genes at undesired loci of the human genome in cells. The ZFN-based strategies could provide an efficient and effective means of directly and specifically editing "defective" bases in genes or provide functional protein complementation by targeted insertion of the wild-type therapeutic gene at a safe harbor locus within the human genome, making gene correction and functional protein complementation of a defective gene viable options. It has the potential to dramatically change the field of gene therapy. Precisely targeted genetic engineering of SCD hiPSCs cannot be done effectively by current routine technologies. This limits laboratory research in human cells and potential translation to pluripotent stem cell-based clinical therapies. The focus of this proposal is to continue my current research to investigate the efficacy and specificity of ZFN- (or TALEN-) mediated gene targeting in SCD hiPSCs. If ZFN-based strategies are successfully applied for targeted engineering of the genome of SCD hiPSCs, it will signify a major advance not only for laboratory research but also to translational clinical applications using disease-corrected hiPSC-based medical therapies. This research has the potential to contribute to the development and expansion of stem cell research in Biotechnology in Maryland and medical therapies to treat monogenic diseases.

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

Scleroderma is a disabling condition characterized by inflammation, blood vessel damage, fibrosis of skin and internal organs, and an immune response to a variety of cellular proteins. While disease manifestations vary among individuals, patients affected by the more severe forms of scleroderma have widespread organ involvement, significant disability, and death within a few years. We do not currently have a good understanding of what causes this disease or how it can be prevented, controlled, or cured. Blood vessel damage occurs early in scleroderma and is ongoing with progression of the disease. While blood vessel injury is usually associated with repair and healing, evidence shows that the vessel repair process is abnormal in scleroderma. In affected tissues of patients with the disease, blood vessel loss and abnormal regeneration are observed, suggesting that the immune response might be directed at the healing blood vessel, and that whenever healing is attempted, additional damage becomes focused on the blood vessel wall. Based on our preliminary data, we believe that differentiating stem cells contain high levels of unique proteins which are targeted by the immune system in scleroderma patients. Such proteins make differentiating blood vessel cells the targets for immune destruction, necessitating more blood vessel repair and more damage in an amplifying injury cycle. We plan to stimulate stem cells to generate blood vessels. We will then use serum from several hundred patients with scleroderma and healthy controls to define whether patients recognize proteins specifically found in these cells compared to a standard mature cell source. We will see if there is a difference in the proteins recognized by patients compared to healthy controls between cell types (stem vs. mature). We believe that the antibodies from scleroderma patients, unlike those from the healthy controls, will recognize unique proteins in the stem cells that are not present in the standard cells. This would suggest that the proteins of interest may have a role in the disease. Consequently, we will also screen the aforementioned cells with blood from patients with other autoimmune conditions to see if the recognition of the unique proteins is specific for patients with scleroderma, or if it is common among patients with a variety of autoimmune diseases. Through this project we will develop a better understanding of the underlying mechanisms of scleroderma and possibly other autoimmune conditions. Our studies will enhance the current knowledge of stem cell biology. Subsequent findings will potentially influence new approaches to diagnosis, monitoring, and treatment.

KARA SCHEIBNER
University of Maryland, Baltimore (UMB)
Awarded Budget: $230,000.00
Disease Target: Hematopoietic Disorders



WENXIA SONG
University of Maryland College Park
Awarded Budget: $230,000.00
Disease Target: Multiple

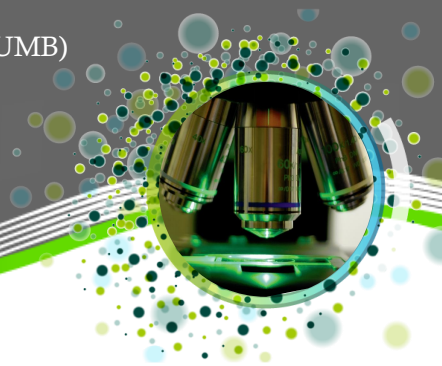
Regulation of DNA Double Strand Break Repair in Human Hematopoietic Stem Cells by MicroRNAs

In order to use blood stem cells in translational applications, several criteria must first be met: 1) there needs to be a significant number of cells, 2) we need to be able to expand these cells as needed, and 3) the cells must be highly functional and maintain their genomic integrity. Double strand breaks (DSBs) in the DNA of a cell can be caused by multiple insults both external and internal, and include gamma-radiation, ultra violet radiation, and highly reactive oxygen species produced by the cell itself. These breaks in both strands of a region of DNA are the most lethal form of damage, and if left unrepaired or are repaired with errors, can lead to cell death or instability of the genome respectively. These DSBs are particularly dangerous in HSCs and progenitor populations; cell death from unrepaired DBSs can reduce the number of HSCs, and as HSCs repopulate the entire blood system, unrepaired damage can be translated to mature blood cell populations. Ultimately, these events can lead to HSC exhaustion or functional deficiencies in mature blood cells. Recent work has suggested different responses of blood stem and progenitor cells to DSBs, dependent on their current state; these populations can be quiescent, meaning that they do not undergo frequent divisions, or, in crisis, they become proliferative to repopulate the system. We still have many gaps in our understanding of this important process in HSCs. This proposal aims to look at both early and late HSCs and progenitors that have been exposed to gamma radiation and determine differences in regulation of the DBS repair response between these populations. One aspect we are particularly interested in is the role of microRNAs (miRs); miRs are short pieces of RNA that bind genes and degrade/destabilize the mRNA transcript or block their translation to proteins. Expression of miRs changes in response to insults like gamma radiation. We will determine if miRs expressed in HSCs and progenitor populations are altered in response to DSBs induced by gamma irradiation, and also determine whether these altered miRs play a role in regulating critical proteins involved in DNA repair pathways and in regulating DSB repair itself. Successful completion of these studies will allow us to potentially use miRs to reprogram HSC and progenitor populations away from cell death and towards a mechanism of DSB repair that maintains genomic stability and functional capacity. Basically, we are attempting to gain insights as to how to "build a better stem cell" for use in translational applications to accomplish a long standing research goal of being able to expand the HSC and progenitor populations to an infinite capacity.

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

B-lymphocytes are a type of white blood cells responsible for generating antibodies and regulating other branches of the immune system. Antibodies protect us by neutralizing pathogens and toxins and by flagging foreign substances and microbial pathogens for immune clearance. Genetic and functional abnormalities of B-cells cause severe diseases, including immunodeficiencies, cancers and autoimmune diseases. X-linked agammaglobulinemia (XLA) is an inherited B-cell deficiency, primarily caused by the mutations of a protein, named as Btk. XLA patients have very few B-cells and no antibodies. Consequently they suffer repeated infections and require regular intravenous immunoglobulin infusions. In contrast, non-Hodgkin lymphoma patients experience the uncontrolled proliferation of B-cells in their lymph tissues. Development of effective treatments for these and other B-cell-related diseases is hindered by a limited mechanistic understanding and a dearth of adequate research models. Currently, the most effective treatment available for many of these diseases is bone marrow transplantation. However, this treatment is associated with severe side effects, including graft-versus-host disease and severe infections, caused by donor-patient incompatibility and immune suppressive treatments required before and after transplantation. In 2006, Shinya Yamanaka made the revolutionary discovery that enables the reprogramming of human adult somatic cells into pluripotent stem cells (hiPSCs). These hiPSCs have the potential to differentiate into multiple cell lineages, thereby providing patient-specific cell sources for transplantation and gene therapies as well as for modeling human diseases. The clinical and research application of hiPSC in B-cell-related diseases, such as XLA, requires an efficient way to differentiate hiPSC into B-cells outside of human bodies. My laboratory has been studying the cellular mechanisms controlling B-cell activation for last 15 years and has contributed significantly to the understanding of the role of Btk in B-cell-mediated immune responses. Our long-term goal is to understand the mechanistic causes of B-cell-related human diseases using hiPSCs as the model. We will explore new methods and technologies for efficiently differentiating hiPSCs into B-cells and characterize the properties and functions of these B-cells. We will generate B-cells similar to those in XLA patients by altering the activation and expression levels of Btk in hiPSCs using chemical inhibitors and genetic manipulations. The success of the proposed studies will provide the first hiPSC-based model for studying B-cell-related human diseases and build a foundation for future applications of hiPSCs in cell therapies of B-cell deficiencies and B-cell cancers.

JOSEPH STAINS
University of Maryland, Baltimore (UMB)
Awarded Budget: $229,412.00
Disease Target: Osteoarthritis



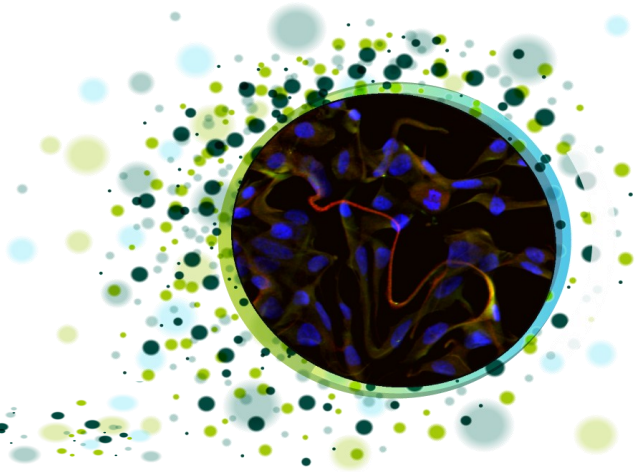
BHANU TELUGU
University of Maryland College Park
Awarded Budget: $228,259.00
Disease Target: Cancer

Using Gap Junctions to Enhance Stem Cell Therapies in Osteoarthritis

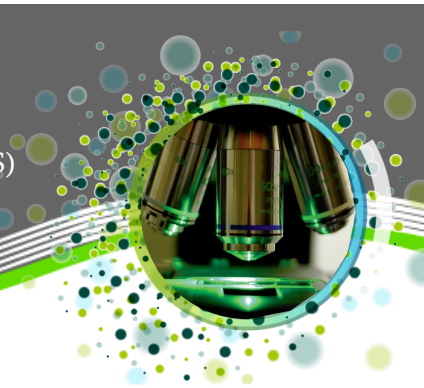
Osteoarthritis is a debilitating disease with no cure. While stem cell based therapies hold great potential for treating degenerative diseases like osteoarthritis, the cells must first overcome the destructive environment of the joint before the cells can reverse the disease. One possible route of controlling the joint environment is though gene therapy. However, like stem cell therapy there are many limitations. In this application, we propose combining stem cell and gene therapies into a single delivery method for modifying the osteoarthritic joint. We propose to use human mesenchymal stem cells (hMSC), which have the ability to repair joint tissues, and "load" these cells with the vector for gene therapy. The advantage of this approach is that the gene therapy vector is masked from the immune response, degradation and diffusion out of the target tissue by being packaged in the hMSC. Upon injection into the joint, we propose that the hMSC can form a communicative gap junction channel with the articular chondrocytes and synovial fibroblasts that contribute to osteoarthritis. Upon forming this channel, we predict, based on published studies, that the gap junctions will permit the gene therapy effector - in the form of short inhibitory RNAs - to pass directly from the hMSC to the host tissue without exposure to the extracellular environment. The result is a directed targeting and inhibition of destructive factors produced by the host cell. When these destructive factors are thus inhibited, the hMSCs are able repair the damaged articular cartilage, potentially reversing the joint damage. We will use molecular and cell biological techniques to test the ability of the hMSCs to be retained in the joint, the ability of hMSCs to form functional gap junction channels with the host tissue and the ability of hMSC to effectively deliver siRNAs to pathologic host tissue. Ultimately, we hope to use this approach to attempt to slow, halt or reverse osteoarthritis progression.

Role of Crucial Oxygen Sensitive Transcription Factor, HIF1A in Stemness & In Disease

Hypoxia inducible factor 1alpha (HIF1A) is an oxygen sensing transcription factor that regulates the expression of a subset of genes within the cell in response to low physiological oxygen concentrations (hypoxia). HIF1A has been shown to respond to physiological hypoxia by altering cellular energy utilization, promoting vascular supply, etc. However, the specific role of HIF1A in the regulation of pluripotency and the consequence of its persistent activity in precursor stem cells as they differentiate into daughter lineages has not been investigated yet. These topics are of important because, the counterparts of the embryonic stem cells (ESC) in the embryo and the adult stem cells reside in hypoxic environments within the individual. The submitted proposal will investigate two facets of HIF1A function. In the first topic, the function of HIF1A in promoting pluripotency, which is the ability to give rise to multiple cell types upon differentiation will be investigated. A second distinct topic is to determine whether persistent HIF1A expression results in defective differentiation of human ESC into trophoblast cells, the predominant cell type of the human placenta. This latter topic is of high relevance to the underlying pathologies associated with preeclampsia, a human specific pregnancy disorder. In summary, both the research topics address important issues associated with the stem cells and as a consequence are of high significance to the goals and mission of the MSCRF.



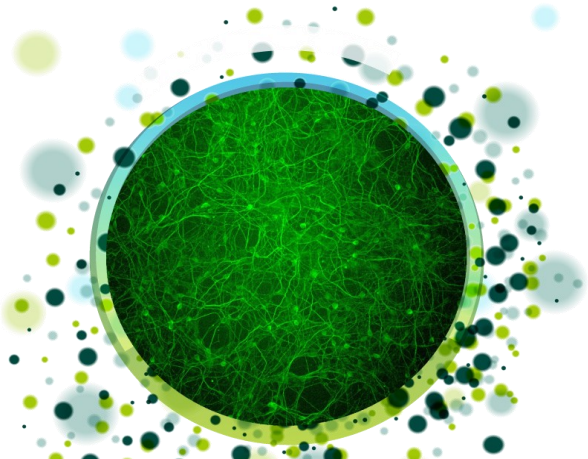
ARUN VENKATESAN
Johns Hopkins University (JHU)
Awarded Budget: $230,000.00
Disease Target: Multiple Sclerosis (MS)



MINGYAO YING
Hugo W. Moser Research Institute at
Kennedy Krieger, Inc.
Awarded Budget: $230,000.00
Disease Target: Parkinson's Disease

Novel Human iPSC-Based Model Of Axon Degeneration In Multiple Sclerosis

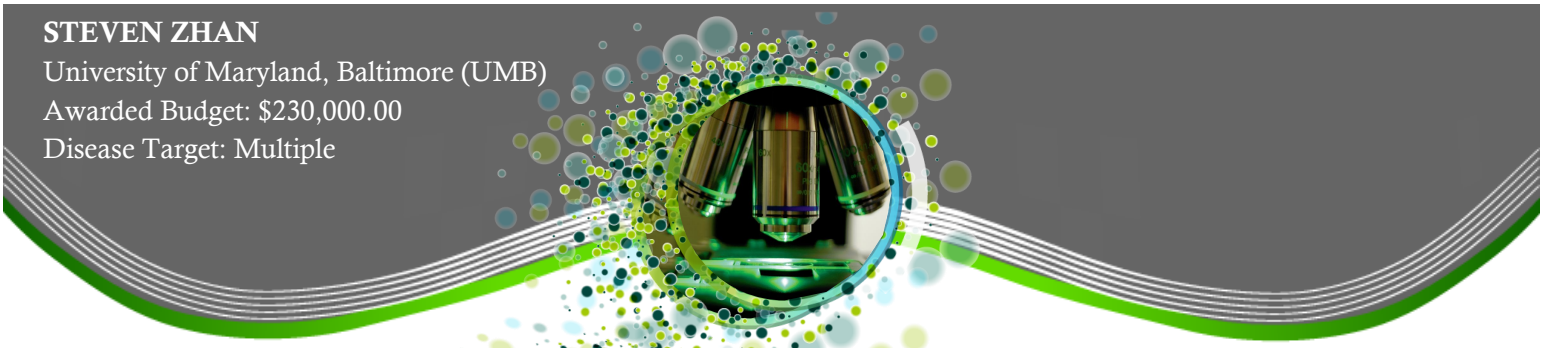
MS is a chronic neurodegenerative disorder of the central nervous system (CNS) of unknown cause that affects 400,000 individuals in the U.S., and is one of the leading causes of disability in young and middle-aged adults. MS has traditionally been viewed as an autoimmune disease in which the body's immune system attacks its own nervous system. This results in stripping of myelin, the insulating material surrounding the cable-like axons which connect one nerve cell to another. Once the myelin is stripped off, it is thought that the axonal cables are more susceptible to inflammatory damage and degeneration, thus resulting in disability. Recent studies have suggested, however, that axon damage occurs early in the disease and that it may precede damage to myelin and other nervous system components. Here, we propose to use new stem-cell based models to investigate this novel hypothesis of MS and to identify drugs that can protect axons from damage. Overall, we anticipate that the use of novel stem cell approaches proposed here will shed new light on the causes of MS and will identify new therapies for affected individuals.



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

Cell replacement therapy using human pluripotent stem cells holds promise for neurological diseases with a relatively selective cell loss, such as Parkinson's disease, in which dopaminergic neuron degeneration is responsible for motor symptoms in patients. Dopaminergic neurons generated from human induced pluripotent cells provide patient-specific cell source for biomedical research and cell replacement therapy. However, current strategies for generating dopaminergic neurons from human stem cells generally yield incomplete dopaminergic neuron differentiation, which adversely affects the function of these neurons and also leads to considerable safety concerns regarding their potential for tumor formation. We have identified transcription factor Atoh1 as a potent neuronal differentiation inducer with greater than 90% efficiency in human neural stem cells. When combined with the morphogen Sonic Hedgehog, Atoh1 drives the differentiation of human neural stem cells into dopaminergic neurons with more than 80% efficiency, suggesting Atoh1-based translatable applications for generating neuronal cells (e.g. dopaminergic neurons) for cell replacement therapy and disease modeling. In this project, we propose to develop an Atoh1-based differentiation strategy for efficiently generating functional dopaminergic neurons from human induced pluripotent cells and embryonic stem cells. We will further investigate the therapeutic potential of transplanting Atoh1-induced dopaminergic neurons in Parkinson's disease animal models. The goal of this research is to establish an Atoh1-based protocol to achieve dopaminergic neuron conversion of >80% of total cells. If successful, we expect to establish a highly efficient Atoh1-based strategy for generating functional dopaminergic neurons that show therapeutic effect in Parkinson's disease animal models. These studies will provide essential information toward the successful application of Atoh1 in cell replacement therapy for Parkinson's disease and other neurological disorders. It will also establish broadly-applicable strategies for evaluating and translating other transcription factors for lineage-specific regenerative therapy.

STEVEN ZHAN
University of Maryland, Baltimore (UMB)
Awarded Budget: $230,000.00
Disease Target: Multiple

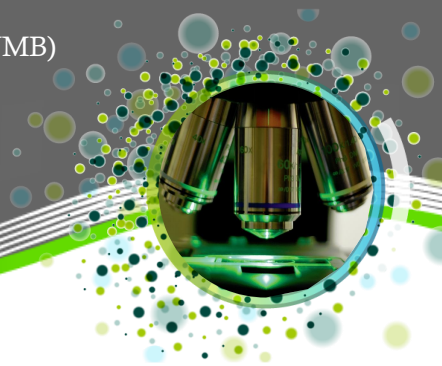


Modulation of Homing and Engraftment of Hematopoietic Stem Cells by I-BAR Proteins

Patients with leukemia or lymphomas often bear blood cells that are defective in normal functions, which may eventually lead to death if untreated. One of the effective clinical approaches to restore normal blood cells of such a patient is through transplantation of blood or hematopoietic stem cells derived from healthy donors into the patient. This approach is possible because the transplanted stem cells are able to travel to the bone marrow of the recipient where they can be further developed into a variety of mature blood cells. However, the current procedure of hematopoietic stem cell transplantation has limited success and is frequently associated with severe side effects. At least part of the reason for the side effects is that the transplanted cells may not interact properly with the bone marrow of the patient who receives the cells. Current drugs are mainly used to increase the mobilization of hematopoietic stem cells from the bone marrow but do not improve the traveling of transplanted cells into the bone marrow. Therefore, there is a clinical need to explore new factors that control the travelling of hematopoietic stem cells and their subsequent interactions with the host bone marrow environment. The main subject of this application is a gene called MIM, which is a member of the inverse-BAR family and regulates the architecture of cell membranes. Because cell membranes are associated with many proteins necessary for the interaction with factors circulated in the blood or on the surface of other cells, changes in the membrane structure could have a great influence on the fate of transplanted cells and their communication with local environment. Indeed, we have recently generated a mouse strain in which the MIM gene had been disrupted, and found the most striking defect with these mice is that blood cells and their early forms were unable to migrate properly in response to signals from local environment. Thus, we hypothesize that MIM is involved in the trafficking of human hematopoietic stem cells. We also think that there is a possibility that one may control the response of transplanted cells to the host microenvironment through modulating the function of MIM protein. These hypotheses will be tested by two sets of experiments described in this application. The first one is to define whether MIM is able to control the trafficking of human hematopoietic stem cells after transplantation; and the second one is to discover drugs that can modulate the function of MIM protein either negatively or positively. Because the function of MIM is significantly different from the targets used by the existing hematological drugs, we expect that the success of this application will have a great impact on the current therapies based on hematopoietic stem cells.

POST-DOCTORAL FELLOWSHIP ABSTRACTS

OLA AWAD
University of Maryland, Baltimore (UMB)
Awarded Budget: $110,000.00
Disease Target: Gaucher's Disease



AMNON BAR-SHIR
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Multiple

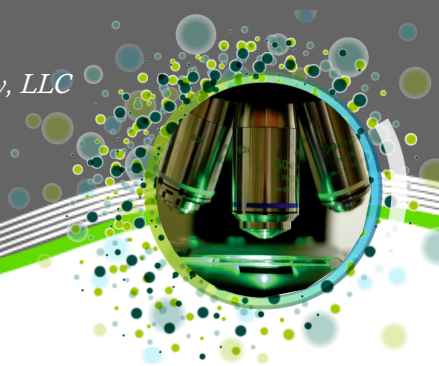
Role of Autophagy Dysregulation in the Development of Neurodegeneration Using iPSC Model of Gaucher's Disease

Autophagy is an essential process for cellular health and survival in particular in the nervous system, where accumulation of protein aggregates and other cellular debris are linked to the development of many different neurodegenerative disorders. While autophagy dysregulation is known to be involved in the neurodegenerative process, the precise mechanisms by which abnormal autophagy lead to neuronal loss is unknown. One major limitation to elucidate such a mechanism is the difficulty to obtain and culture the relevant neuronal cell types carrying patient-specific mutations. We will use induced pluripotent stem cells (iPSC) from patients with neuronopathic disease due to mutations in the glucocerebrosidase gene, to investigate the molecular mechanisms involved in neuronal loss. We have already generated and characterized Gaucher disease-specific iPSC derived from all the three clinical types including the neuropathic forms type 2 and type 3. We showed that the disease-specific iPSC are pluripotent and can efficiently differentiate into mature post-mitotic neurons. Our goal is to use Gaucher iPSCs-derived neurons to study autophagy-dependent mechanisms responsible for the neuronal loss, as a model for neurodegenerative disorders. Our preliminary studies and evidence from the literature indicate that there is dysregulation in cellular autophagy in Gaucher's disease, which may be due to the inherited lysosomal dysfunction. We hypothesize that autophagy perturbation is central to neuronal loss in neuropathic form of Gaucher's disease. Using cellular and molecular techniques, we will use iPSC-derived Gaucher neurons to investigate the precise mechanisms underlying autophagy dysregulation and how it mediates neuronal loss. We will also use known compounds capable of blocking or enhancing various components of autophagy pathways and test their effects on neuronal survival in mutant- vs. control iPS-neurons. Our aim is to identify new therapeutic targets and chemical agents able to promote neuronal survival and prevent neurodegeneration in Gaucher patients. We believe that this research will provide fundamental knowledge applicable to the role of autophagy dysfunction in other neurodegenerative diseases as well.

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

The ability of stem cells to self-renew, and to differentiate into many downstream phenotypes, makes them ideal candidates for the treatment of a variety of diseases as diverse as neurological conditions or cancer. The principle of regenerative medicine and the use of stem cells is based on their potential to mature, form functional derivatives, integrate with host tissues, and ultimately, repair or replace damaged cells or tissues. Therefore, stem cell transplantation is a promising strategy to target and treat various diseases. However, one of the primary critical concerns in stem cell transplantation is the substantial loss of viable transplanted cells after transplantation into hostile, disease-damaged tissues. Various hydrogel-based biomaterials have been employed to improve the survival of engrafted cells by introducing a unique three-dimensional microenvironment. Although the effect of the hydrogel embedding on the survival of stem cells has been studied in culture, few reports exist that characterize its supportive properties in vivo. A robust, non-invasive method for monitoring such scaffold-seeded stem cells will allow the assessment of graft viability and functionality in real-time. Such a technique would have a significant impact on optimization and improvement of hydrogel scaffolds for the effective support of stem cells. We propose an innovative approach to elucidate the fate of stem cells seeded in scaffolds using an MRI-based reporter gene. The viability of the transplanted progenitor cells expressing the HSV1-tk reporter gene will be monitored longitudinally and simultaneously with the dynamic structural changes of the embedding hydrogel. Upon completion of this study, we anticipate (i) having established a new approach for the non-invasive imaging of transplanted cells, and (ii) using that technique to optimize the chemical and mechanical properties of their seeding scaffold. In the future, we will extend this new approach for imaging the functionality of transplanted cells and their migration capabilities. Although introducing the genetically encoded reporters is currently restricted to preclinical studies, we anticipate that, with expanding knowledge and safety for clinical applications of gene therapy in the future, such reporters could be applied in patients.

SU MI CHOI
Johns Hopkins University (JHU)
In collaboration with Cellomics Technology, LLC
Awarded Budget: $110,000.00
Disease Target: Liver Cirrhosis



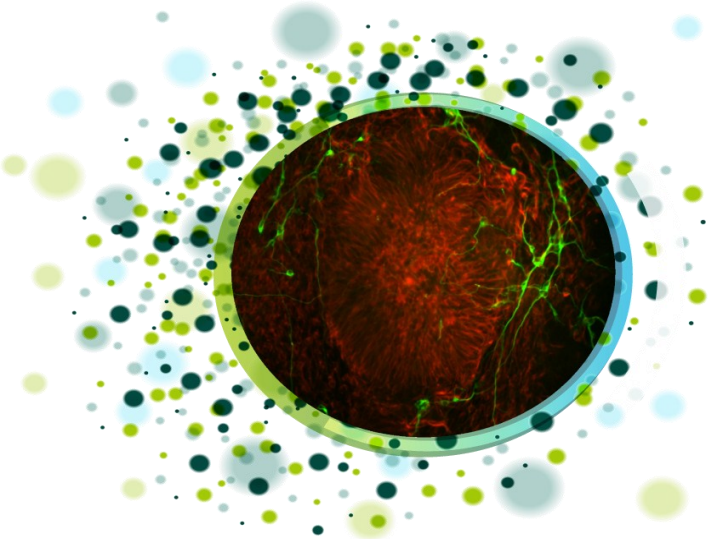
CHRISTOPHER DONNELLY
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: ALS

Patient Specific Stem Cell based In Vitro Model of Liver Cirrhosis

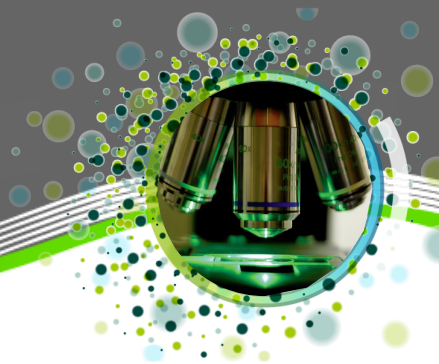
Liver fibrosis will eventually lead to liver cirrhosis which is one of the top ten causes of death in the world. Currently there is no treatment for preventing fibrosis progression or reversing fibrosis/cirrhosis. Liver transplantation can be done for only a limited number of the end stage liver failure patients depending upon organ availability. Moreover, there is lack of human relevant in vitro or in vivo liver cirrhosis models to study and develop therapy for cirrhosis. Current animal based models cannot accurately represent human pathophysiology and primary human hepatocytes from cirrhosis patients are limited by their inaccessibility. There have been no human cell lines established from cirrhosis patients unlike neoplastic diseases where human cancer cell lines are frequently available for disease mechanism studies and drug screening. The emerging induced pluripotent stem cell (iPSC) technology provides an alternative for generating functional, renewable and relevant cell sources for disease modeling using patient tissues as we have recently shown with an inherited liver disease. In addition, we have established patient iPSC lines from diverse liver diseases including liver cirrhosis and those with potential to progress into liver cirrhosis (e.g., alcoholic or viral hepatitis, alpha-1 antitrypsin deficiency, and hemochromatosis). Taking advantage of established iPSC lines and liver disease modeling systems already established in our laboratory, I propose to establish an in vitro model of liver cirrhosis. Since liver cirrhosis has a complex pathogenesis involving multiple cell types and causes, we plan to use a multiparametric approach to capture and assess the cellular pathology for disease modeling. Both the epithelial mesenchymal transition of hepatocytes and the activation of hepatic stellate cells (HSCs) have been shown to play important roles in liver fibrosis and cirrhosis. Towards establishing an in vitro liver cirrhosis model focusing on recapitulating the initial events in liver fibrosis, we will evaluate the multiple aspects of fibrotic changes for reliable readout. We will test the hypothesis that human iPSCs will provide effective tools for modeling liver cirrhosis in vitro, which could provide a molecular understanding of the disease process as well as enable the discovery of novel therapies for many liver cirrhosis patients without therapeutic options.

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

The C9ORF72 hexanucleotide repeat expansion has been identified in ~30% of familial and ~4-10% of sporadic ALS cases and is therefore the most common genetic abnormality associated with ALS to date. Repeat expansions in non-protein coding regions are the known cause of other neuromuscular disorders (e.g., DM1/2, FXTAS) and pathogenicity is thought to arise from aberrant binding of trans acting factors to the cis repetitive elements. This is best studied in DM1 where MBNL1 is sequestered by the 'CTG' expansion in the DMPK pre-mRNA. Therefore, antisense oligonucleotide (ASO) and RNAi (siRNA) strategies designed to block binding to the cis elements may have therapeutic potential in these disorders. To test this possibility in the context of C9orf72 ALS, we designed siRNA and ASO methodologies to target the C9ORF72 transcript and repeat expansion, and have demonstrated knockdown the C9ORF72 gene in patient-derived cell lines. Furthermore, utilizing our transcriptome profiling generated from C9ORF72 ALS patient fibroblasts, iPS derived CNS cells and validated with human autopsy tissue, we show that ASO/RNAi treatment can normalize specific genes which are aberrantly expressed in cell lines that contain the 'GGGGCC' expanded repeat. These approaches will allow us to effectively develop 1) antisense mediated therapeutic approaches to ALS and 2) relevant pharmacodynamic readouts for antisense efficacy.



GABRIEL GHIAUR
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Schizophrenia



PINAR HURI
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Bone Disease

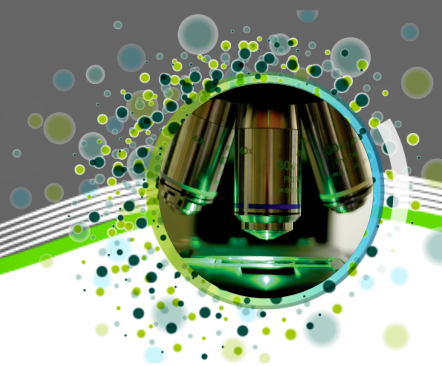
Retinoic Acid (RA) Controls Self Renewal & Differentiation of Human Hematopoietic Stem Cells (HSCs)

The process of hematopoietic stem cell (HSC) self-renewal, although critically important both biologically and clinically, remains poorly understood. Since retinoic acid (RA) induces terminal differentiation of both normal and leukemic hematopoietic progenitor cells in vitro, we hypothesize that RA signaling is at least partly responsible for the loss of HSCs during culture. Accordingly, we further hypothesize that inhibition of RA signaling will produce HSCs expansion in culture. In addition, the microenvironment in which HSCs reside is essential to maintain the balance between their self-renewal and differentiation both in vivo in the stem cell niche, as well as in vitro during Dexter type culture. Clinical advances in blood and marrow transplantation (BMT), gene therapy and regenerative medicine have been hampered by the scarcity of HSCs and the inability to maintain and expand these cells in vitro. Although HSCs possess the capacity to self-renew in vivo, expansion/ maintenance of these cells in vitro could be considered the Holy Grail of hematopoiesis. Thus, we propose to better understand the fate decision between self-renewal and differentiation of individual HSCs with the promise for future clinical advances in stem cell therapies. HSCs reside in a highly complex niche that assures their survival and presumably allows their self-renewal. Disruption of these interactions, as occurs during in vitro culture, results in cell cycle activation, differentiation and rapid loss of HSC activity. Similarly, the microenvironment provided by various stroma cell lines has been the best way to maintain HSCs activity in vitro. Understanding how stroma maintains HSC activity may provide the tools needed for clinically relevant expansion of human HSCs in vitro. HSC self-renewal is regulated by an intricate network that integrates intrinsic and extrinsic signals. The current project addresses how these mechanisms are affected during in vitro culture. RA has been shown to be important in Scl/Tal1 and AHR signaling, Hox genes patterning, and FoxO expression, to name just a few cell intrinsic mechanisms that control HSCs fate. Here we will investigate to what extent these pathways are modulated by inhibition of RA signaling in human HSCs. In addition, while RA has been studied as a cell intrinsic factor to promote differentiation in hematopoietic cells, we will also investigate if the microenvironment controls HSCs fate by modulating the availability of RA to the HSC compartment.

Engineering Clinically Applicable Vascularized Bone Grafts Using Adipose Derived Stem Cells

Tissue engineering (TE) could be an ideal solution to the worldwide shortage of bone substitutes for clinical implantation. One of the current challenges of TE is the lack of proper cell sources and adipose-derived mesenchymal stem cells (ASCs) are gaining increased attention as a multipotent, easily accessible and abundant cell source that makes it amenable for autologous use. The development of strategies that could effectively induce the establishment of a microcirculation in the engineered constructs has become a major goal for tissue engineering. A recent strategy to provide a solution involves the development of multi-functional scaffolds capable of delivering the required bioactive agents to regulate the activities of implanted and host cells. Development of such complex constructs, which induce graft vascularization as well as osteogenesis, would greatly enhance the translation potential of tissue engineered bone grafts into clinical applications. In this study, the aim is to produce functional, anatomically-shaped, vascularized tissue engineered bone substitutes using ASCs as a single cell source to give both vascular and osteogenic compartments through the spatio-temporally controlled release of growth factors. Polymeric, growth-factor-laden nanocapsules (NC) incorporated within porous, mechanically strong scaffolds will provide biological cues that guide the differentiation of ASCs and enable healing of critical-sized bone defects. 3-D scaffolds are prepared from poly(ε-caprolactone) (PCL), a completely biodegradable, thermoplastic polyester suitable for use in long term load bearing applications. Currently the effect of internal PCL structure on the controlled release of fibroblast growth factor-9 (FGF9) and bone morphogenetic protein-2 (BMP2) to induce perivascular and osteogenic differentiation of ASCs, respectively, are being tested with a wide range of pore sizes ranging from 50-500 μm. The effect of PCL internal architecture on the proliferation and differentiation of ASCs are also being studied. Meanwhile, the temporal responses of ASCs to the controlled delivery of FGF9 and BMP2 - singly and in combination - to produce vascularized bone grafts are studied. These studies will ultimately lead to the development of anatomically-shaped well characterized biodegradable bone grafts with the capability to deliver multiple growth factors in a spatiotemporal fashion to induce vascular and osteogenic differentiation of ASCs. Overall, the approach will enable the development of easily translatable, patient-specific, vascularized bone grafts capable of treating bone defects.

EUNCHAI KANG
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Schizophrenia



CHAEKYU KIM
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Musculoskeletal Systems

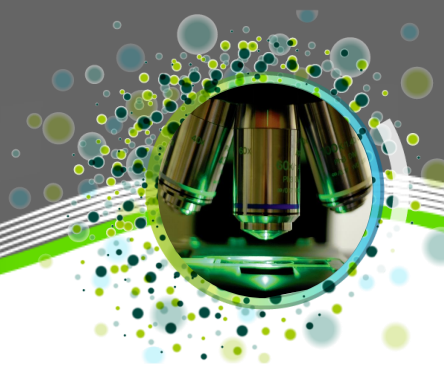
Modeling Of Major Mental Disorders Using Human Induced Pluripotent Cells Derived From Patients With A Defined Disc1 Mutation

Schizophrenia (SCZ) is a devastating mental disorder with a prominent genetic basis. Many candidate genes associated with increased risk for SCZ have been identified that appear to support a neurodevelopmental origin for this disorder. Disrupted-in Schizophrenia1 (DISC1) is a prominent SCZ susceptibility gene originally identified at the break point of a balanced chromosome translocation t(1:11)(q42;q14), which co-segregates with major mental disorders, such as SCZ, in the large Scottish pedigree. Many genetic studies have since confirmed the association of DISC1 with SCZ in various populations. Emerging evidence from rodent studies has shown that DISC1 regulates various aspects of neurodevelopment including proliferation, maturation and integration of newborn cells into the existing circuitry at different time points. However, there are significant differences in development and structure between the rodent and human brain as well as interspecies differences in DISC1 at both the gene and protein level. Currently, the function of human DISC1 is poorly understood. Induced pluripotent stem cells (iPSCs) derived from skin fibroblasts of SCZ patients with a DISC1 mutation provide a unique model system to study the role of DISC1 in human neurodevelopment. The goal of the current study is to understand the biological function of human DISC1 in neurodevelopment, and identify the molecular mechanisms underlying SCZ using a pharmacological approach with two aims: 1) to characterize the biological function of human DISC1 in neurodevelopment using human neural progenitor cells (hNPCs) derived from schizophrenia patients with a DISC1 mutation in vitro and in vivo 2) to identify genetic and pharmacological approaches to rescue the developmental defects identified in human neurons with a DISC1 mutation. The proposed study will be an important entry point toward understanding the biological function of human DISC1 and deciphering the molecular mechanisms underlying the pathogenesis of SCZ. The proposed in vivo humanized animal model to examine the impact of this disease risk gene in regulating human neuronal development under in vivo conditions may serve as a preclinical model for testing novel therapeutic treatments in the future.

Small Molecules to Influence Metabolism in Stem Cells &Tissue Formation in Hydrogels

Tissue engineering and regenerative medicine are starting to make inroads into clinical practice and changing the way medicine and surgery address tissue loss from trauma, disease, or congenital abnormalities. However, there is potential to expand the application of tissue engineering beyond replacement and restoring native tissue such that we can engineer tissues with unexpected functions and therapeutic capacity to treat systemic disease. Synthetic biology is an emerging field that aims to build biological circuits of simplified genetic modules that perform a desired function. The technology has recently been applied to mammalian cells where systems have been engineered to turn genes on and off in a highly regulated and tunable manner. For example, synthetic circuits have been created to regulate genes through inducers such as light or small molecules. When exposed to the stimulus, the gene that is regulated by the genetic circuit turns on and when the stimulus is removed the gene turns off. We aim to incorporate these simple circuits into progenitor cells and build tissues with new capabilities by combining tissue engineering with synthetic biology to create Smart Tissues with tunable therapeutic functions. Mesenchymal progenitor cells are key building blocks of many tissues including bone, fat, and cartilage. We, along with many researchers, have employed biomaterials in combination with progenitor cells to create new tissues, some of which have reached clinical testing. Here, we investigate the application of synthetic control switches to progenitor cells that will be used to engineer Smart Tissues with externally controlled genetic response elements. Specifically, the genetic switch, LTRi_EGFP will be incorporated into progenitor cells and bone tissue with normal endocrine functions will be engineered. Green fluorescent protein (GFP) will be used as the model gene that will be turned on and off with the simple sugar inducer IPTG, isopropyl-b-thiogalactopyranoside.

CHANGMEI LIU
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Multiple



GEORGIA MAKRI
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Rett Syndrome

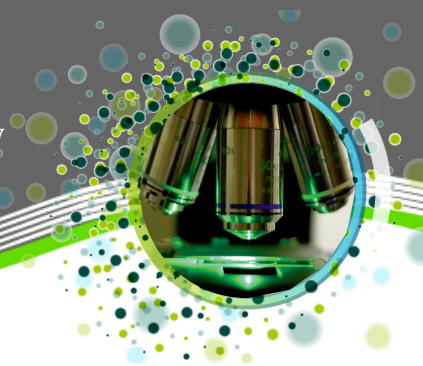
Small RNA Regulation of GSK3 Expression Modulates Human Neural Stem Cells Proliferation & Differentiation

Glycogen synthase kinase 3 (GSK3) has been shown to be associated with many neurological diseases. Inhibition of GSK3 with pharmacological inhibitors is emerging to be a key approach in controlling human embryonic stem cell neural differentiation or generating induced pluripotent stems (iPS) cells. Micro-RNAs (mi-RNAs) are non-coding RNAs that function as epigenetic regulators of a variety of biological processes, including proliferation, differentiation and maintenance of stem cells. Several mi-RNAs, such as let-7, miR-124, miR-9, miR-25, miR-134, miR-137, and miR-184, have been shown playing important roles in NSCs. To determine if GSK3 is regulated by mi-RNAs, we firstly down-regulated Dicer, a key protein for producing mi-RNAs in mouse embryonic NSCs. Our western blot data showed that protein expression level of GSK3α and GSK3β were markedly increased afterDicer knockdown in mouse embryonic NSCs. We then used the software TargetScan 6.1 to predict the potential mi-RNAs that might have binding sites on the 3'-UTR of GSK3α and β. Dozens of mi-RNA are predicted out as potential GSK3's regulators. To test if GSK3 is the direct target of these predicted mi-RNAs, we cloned 3 untranslated regions (UTRs) of GSK3 directly from mNSC's cDNA and generated a dual luciferase 3 UTR reporter construct. Using this reporter, we found that overexpression of miR-92b, miR-221/222 in mouse neural stem cells could suppress the GSK3β 3′ UTR luciferase reporter activity, indicating that these mi-RNAs may directly bind to the GSK3β 3′ UTR and regulate GSK3 expression. In addition, we showed that overexpression of these three mi-RNAs also significantly increased the proliferation of NSCs. As inhibition of GSK3 can promote NSC proliferation, our results suggest that miR-92b and miR-221/222 regulate NSCs proliferation via down-regulating the expression of GSK3. Based on the above experiment results, we will verify if these mi-RNAs still target GSK3 in human neural stem cells (hNSCs) by using the luciferase reporter assay and both gain- and loss-of-function experiments. In addition, we will determine the functions of these mi-RNAs in controlling hNSC proliferation and differentiation. Finally, we will identify if GSK3 acts as a downstream target of these mi-RNAs to regulate the proliferation and differentiation of hNSCs.

Patient-Specific iPSCs for Modeling & Treatment of Rett Syndrome

Rett syndrome (RTT) is a severe autism spectrum disorder causing progressive loss of motor and language skills that primarily affects girls between 6 and 18 months of age. Clinical features include deceleration of head growth, loss of purposeful hand movements, ataxia, loss of vocalization skills, autistic symptoms, seizures and respiratory dysfunction. Mutations in the X-linked gene MeCP2 account as the primary cause of the disease. There is an array of RTT-causing MeCP2 mutations, from missense, nonsense, insertions, deletions, and splice site variations, which are dispersed throughout the gene. Previous studies have shown that increased expression of MeCP2 in hippocampal pyramidal neurons results in a significant reduction in the number of spines, similar to that seen following the loss of MeCP2 function. These data suggest an optimal set point for MeCP2 expression to preserve the integrity of neuronal development. For this study we are going to use iPSCs from patients with RTT to model the disease and try to understand the pathology that leads to RTT. Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) was a breakthrough discovery in 2006 by when it was shown that murine fibroblasts can be reprogrammed by over-expressing four transcription factors: Oct4, Sox2, Klf4 and c-Myc. Reprogramming technology has since been applied to derive patient-specific iPS cell lines, which carry the identical genetic information as their patient donor cells. This is particularly crucial for regenerative cell therapy approaches as differentiated patient-specific iPSCs might be used for autologous transplantation, especially in cases where the disease is caused by degeneration of specific cell types. While iPSC lines have been generated from female Rett patients with MeCP2 mutations, male patients with MeCP2 mutations are very rare because MeCP2 is on the X chromosome and normally mutations elicit early lethality in males. For this study we will generate iPSC lines from male patients diagnosed with RTT with the A140V mutation in MeCP2. This mutation typically leads to less severe phenotypes and provides a unique opportunity to compare male and female patients and to selectively differentiate neural progenitors derived from the patient-specific iPSCs to specific neuronal populations that have been implicated in disease etiology, mainly GABAergic interneurons. Finally, we will evaluate the interaction between neurons with a MeCP2 mutation and the host environment through in utero transplantation of human neural progenitors in the developing mouse brain.

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



KIT MAN TSANG
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Multiple

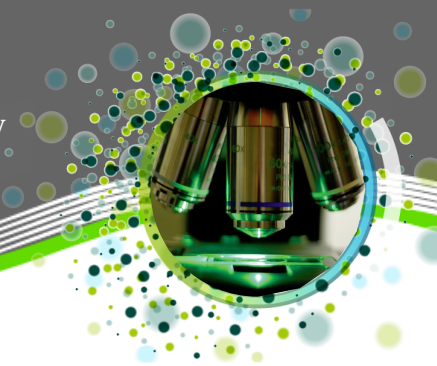
Optogenetic Analysis of Stem Cell Integration into Forebrain Circuits Following Traumatic Axonal Injury

There are about 1.7 million new cases of traumatic brain injury (TBI) in the US every year, most of them from motor vehicle accidents or falls. Although many cases of TBI result in acute concussions, a substantial number lead to chronic disability due to permanent brain damage or, in some cases, progressive neurological disease. Severe forms of TBI include focal contusions often caused by falls, and diffuse axonal lesions (diffuse axonal injury, DAI) usually associated with rotational acceleration as it occurs in motor vehicle accidents. DAI causes widespread axonal denervation at multiple CNS sites including the corpus callosum, brain stem, and grey/white matter junctions of the cortex. Although some circuit plasticity is plausible and pharmacological interventions have some effectiveness, many patients with DAI remain symptomatic decades after injury. The two types of repair tasks for stem cell transplants across TBI forms and models are to replace dead neurons and to support injured neurons, including assistance with axonal repair/regeneration. In this application, we propose optogenetic methodologies to monitor the physiological and behavioral output of transplanted embryonic stem cell (ESC)-derived neuronal precursors (NP) with fiber optics. In such a fashion, we will be able to record physiological responses in both transplanted ESC-derived NPs as well as downstream target neurons and investigate whether these new con-

Epigenetic Regulation of Hematopoietic Differentiation from Human Pluripotent Stem Cells

The pluripotent nature of human induced pluripotent stem cells (iPSCs) opens the opportunity for stem cell-based therapy in regenerative medicine, owning to the anticipation that iPSCs can provide unlimited supply of patient specific cells for autologous transplants and avoid the ethical concerns as in the origin of human embryonic stem cells (ESCs). We and many other investigators have developed various methods to direct ESCs and iPSCs to various lineages of hematopoietic cells in vitro. However, ESCs- or iPSCs-derived hematopoietic cells under current protocols are more embryonic-like that lack long -term in vivo engraftment capability. The importance of epigenetic regulation for both mouse and human HSPCs has been demonstrated in numerous studies. It has been shown that HSPCs have unique epigenetic signature, which correlates to the level of differentiation. A strong association between promoter demethylation and hematopoietic-specific genes expression has also been demonstrated in HSPCs, when compared to ESCs. Although it has been shown that global epigenetic modification can enhance the self-renewal of HSPCs, its functional significance in definitive hematopoietic cells expansion and differentiation from ESCs and iPSCs has not been reported. We conducted a comprehensive DNA methylation analysis not only to compare somatic cells (HSPCs), the corresponding iPSCs and ESCs, but also the derivatives from iPSCs, which are the re-differentiated CD45+CD34+ hematopoietic cells (iBlood). Our study suggested the presence of epigenetic memory from the cell of origin that can alter the differentiation potential of iPSCs. This piece of information suggested that epigenetic memory can be a valuable and useful property to efficiently direct the differentiation of iPSCs towards the lineage of cell origin. In order to identify the epigenetic regulators that can enhance hematopoietic differentiation towards definitive hematopoietic lineage from human iPSCs, engraftable CD34+CD45+ HSPCs from fetal liver (a key site of definitive hematopoiesis in the early fetal stage) will be included in our DNA methylation analysis. Completion of this study will provide useful information on the feasibility in utilizing or modulating epigenome to identify lineage-specific gene(s) or enhance differentiation potential that is critical in the development of specific lineages, hematpoietic lineage in our case, from iPSCs.

YI-LAN WENG
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Traumatic Brain Injury



YAXUE ZENG
Johns Hopkins University (JHU)
Awarded Budget: $110,000.00
Disease Target: Multiple

Effects of DNA Methylation Reprogramming in Axonal Plasticity & Regeneration

Injured neurons regain axonal growth capabilities upon upregulation of a specific set of regeneration-associated genes (RAGs). However, this regenerative ability declines with age and is normally restrained in the peripheral nerve system (PNS) as severed axons of adult CNS neurons are refractory to regenerate. Despite recent studies begun to reveal that regeneration competent state in adult CNS neurons can be maintained under certain circumstances, very little is known about the mechanisms responsible for initiation of regenerative processes. It is therefore critical to address this gap in our knowledge and identify how nerve injury-triggered signals lead to transcriptional activation of regeneration-associated genes, how growth competence is re-established after injury, how this capacity is altered by aging and disease, and most importantly, how it occurs in human neurons. Global changes in gene expression in injured neurons suggest a possible involvement of epigenetic regulation in determining regenerative capacity. A growing body of evidence demonstrates that gene activation via active DNA demethylation plays a crucial role in rapid responses to external stimuli. Furthermore, recent studies, including several from our laboratory have begun to reveal several factors that are involved in active DNA demethylation, including TETs, Gadd45, activation-induced cytidine deaminase (AID)/APOBECs, and thymine DNA glycosylase (TDG). Interestingly, Gadd45a and TET3 are rapid injury-response genes highly expressed in neurons and their expression precedes the elevated levels of regeneration-associated genes. These findings highlight the possibility that Gadd45a and/or TET3 may function as a master regulator of regenerative capacity. Here I will differentiate human induced pluripotent stem cells (iPSCs) into early and later stage motor neurons, recapitulating characteristics of embryonic and adult neurons respectively, to gain insight into age-dependent axon regeneration mechanisms. I hypothesize locus-specific DNA methylation confers hibernation of neurite outgrowth in mature neurons during development. I will identify genes whose epigenetic patterns are associated with axon regenerative capacity by using methyl-sensitive cut counting (MSCC) to create a genome-scale methylation profile and quantitatively compare the methylation landscapes before and after neuronal maturation and with or without axotomy. Additionally, I will introduce Gadd45a and TET family to mature neurons and assess whether forced DNA demethylation can rejuvenate intrinsic growth competence.

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

Abstract and Specific Aims Recent advances in stem cell research have led to the groundbreaking discovery of the ability to reprogram somatic cells to pluripotent stem cells, or induced pluripotent stem cells (iPSCs), by introducing a group of transcription factors [1]. In addition, somatic cells can be reprogrammed directly to neurons, or iNs, without cell division by introducing different sets of transcriptional factors [2]. The advances in reprogramming have tremendous potential for understanding human biology, modeling human disorders, cell replacement therapy and developing new drugs. Although it is known that extensive cellular changes occur during the reprogramming process, the molecular mechanisms underlying these changes are largely unclear. A better understanding of the reprogramming process will lead to more efficient, safer and better reprogrammed cells for translational research. Given the different DNA methylation profiles between pluripotent and differentiated cells [3], identifying the dynamic changes of DNA methylation and associated histone modifications becomes an essential key to unraveling the molecular mechanism of reprogramming. The recent discovery of the active DNA demethylation pathway, TET-APOBEC-TDG [4-7], has provided important clues to characterize key epigenetic events in multiple cellular settings. In this proposal, I will determine the role of active DNA demethylation in the human somatic cell-iPSC conversion and identify their transcriptional targets and associated histone modifications during reprogramming. The goal of the current study is to investigate function of the TET-APOBEC-TDG pathway in reprogramming with three specific aims: Aim 1: Characterize the role of TET-APOBEC-TDG pathway in human somatic-iPSC and somatic- iN conversion. Aim 2: Identify pluripotency/neuronal gene targets of TET1 in reprogramming. Aim 3: Evaluate the crosstalk between DNA modifications and histone modifications on pluripotency genes during the reprogramming process.

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