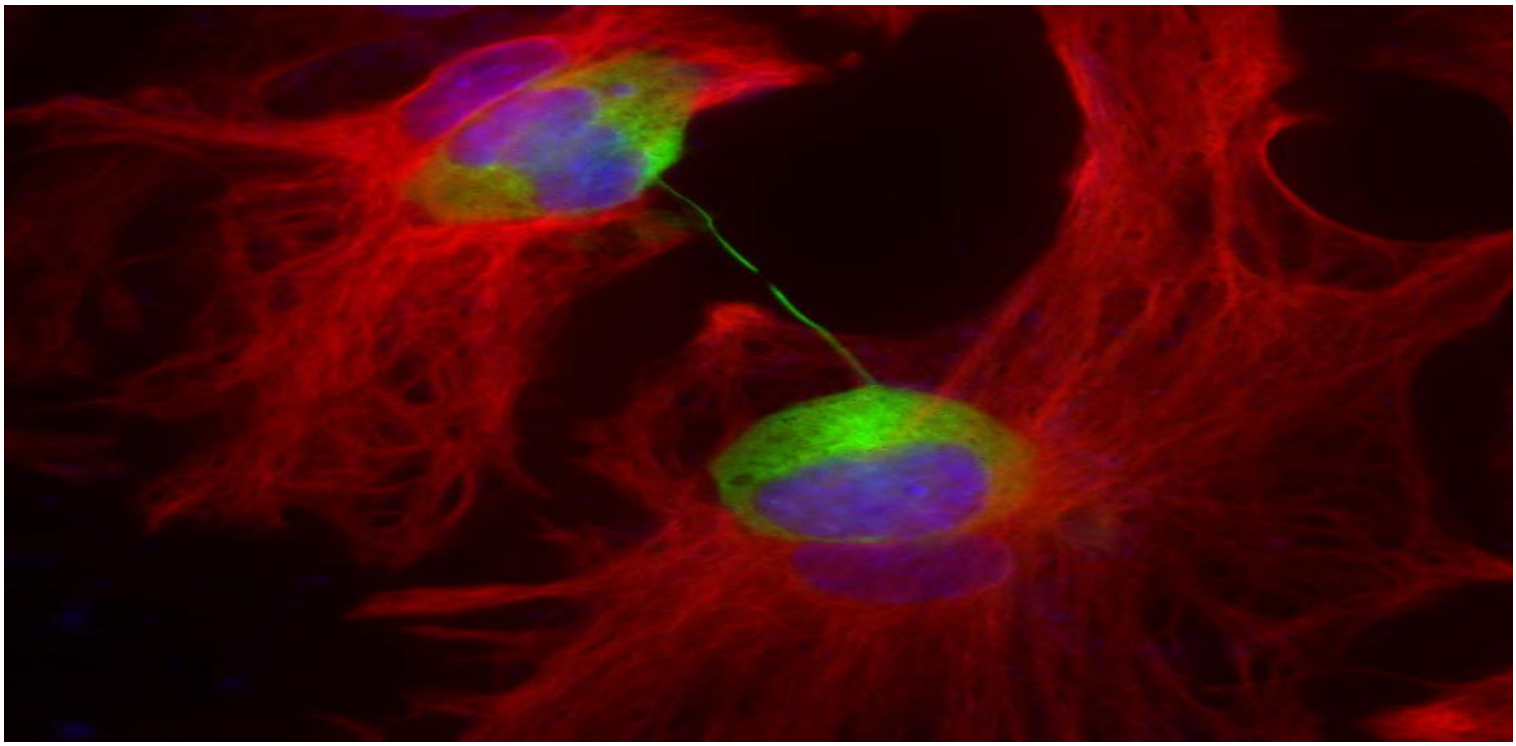


Maryland Stem Cell Commission

2010

Annual Report

Table of Contents

MARYLAND STEM CELL RESEARCH COMMISSION ANNUAL REPORT

2010 RESEARCH ABSTRACTS

TEDCO Board of Directors

Jay Steinmetz, (TEDCO Chair), Barcoding, Inc.
Charles J. "Chuck" Morton, Jr., (TEDCO Vice Chair), Venable LLP.
Jack Lewin, (TEDCO Treasurer), M&T Bank
Lewis C. Powell, (TEDCO Secretary), Antiok Holdings, Inc.
Bill Anderson, Oculis Labs
Dorothy Brunson, Brunson Ross, Inc.
Winston C. Chan, Ph.D., Corvusys, Inc.
Eugene M. Deloatch, Ph.D, Morgan State University
Claire Fraser-Liggett, Ph.D, UM School Of Medicine
Paula L. Jagemann
Christian Johansson , Maryland Department Of Business And Economic Development
Lynn Johnson Langer, Ph.D, Johns Hopkins University
C. Warren Mullins
Eric Orlinsky, Saul Ewing
Amita Shukla, New Enterprise Associates, Inc

~~~~~~

TEDCO Executive Staff

Robert Rosenbaum, President and Executive Director
John Wasilisin, Executive Vice President and Chief Operating Officer

TEDCO-MSCRF Staff

Dan Gincel, Ph.D., MSCRF Director
Sabrina Barksdale, MSCRF Administrative Coordinator
~~~~~~

Commission Members

Maryland Stem Cell Research Commission: This independent Commission functions within TEDCO and consists of the following members:

The Attorney General or Designee:
Ira Schwartz, Senior Assistant Attorney General and Counsel to the Maryland Technology Development Corporation.

Three Patient Advocates:
Bowen P. Weisheit, Jr.(Vice Chair), Board member of the Maryland Chapter of Cystic Fibrosis Foundation and lawyer with the Law Office of Bowen Weisheit, Jr. (appointed by the Governor)

Brenda Crabbs, previous chairwoman of the Maryland chapter of the Arthritis Foundation and member of the organization's Medical and Scientific Council (appointed by the President of the Senate)

Marye D. Kellermann RN, President Educational Entities/Enterprises NECESSARY NP Reviews and NECESSARY Workshops (appointed by the Speaker of the House of Delegates)

Three Individuals with Experience in Biotechnology:
Margaret Conn Himelfarb (Chair), Health Advisory Board and Institutional Review Board, Johns Hopkins Bloomberg School of Public Health; Embryonic Stem Cell Research Oversight Committee, Johns Hopkins School of Medicine (appointed by the Governor)

Linda Powers, Managing Director of Toucan Capital, an early and active supporter of biotech companies (appointed by the President of the Senate)

Dr. Curtis P. Van Tassell, Research Geneticist, USDA-ARS, Beltsville, MD (appointed by the Speaker of the House of Delegates)

Two Individuals Who Work as Scientists for the University System of Maryland and Do Not Engage In Stem Cell Research (Appointed by USM):
Dr. Suzanne Ostrand-Rosenberg, professor of biological sciences and the Robert and Jane Meyerhoff Chair of Biochemistry at UMBC's Department of Biological Sciences

Dr. Steven Salzberg, Director of the Center for Bioinformatics and Computational Biology, and the Horvitz Professor at the University of Maryland, College Park's Department of Computer Science

Two Individuals Who Work as Scientists for the Johns Hopkins University (JHU) and Do Not Engage In Stem Cell Research (Appointed by JHU):
Dr. Sharon Krag, Professor Emerita Department of Biochemistry and Molecular Biology, the Johns Hopkins University Bloomberg School of Public Health

Dr. Noel R. Rose, Director of the Johns Hopkins Autoimmune Disease Research Center, MMI and Pathology, and is the Director of the Pathobiology Training Program in the School of Medicine.

Two Bioethicists, One Appointed by USM and One by JHU:
Karen Rothenberg, Marjorie Cook Professor of Law, founding Director of the Law & Health Care Program, and served as Dean of the University of Maryland School of Law from 1999-2009. (appointed by USM)

Dr. Jeremy Sugarman, Harvey M. Meyerhoff Professor of Bioethics and Medicine, Berman Institute of Bioethics and Department of Medicine Johns Hopkins University (appointed by JHU)

Two Individuals with Expertise in the Field of Biomedical Ethics As It Relates to Religion, Appointed by the Governor:
Rev. Dr. Kevin T. FitzGerald, Associate Professor in the Department of Oncology at Georgetown University Medical Center

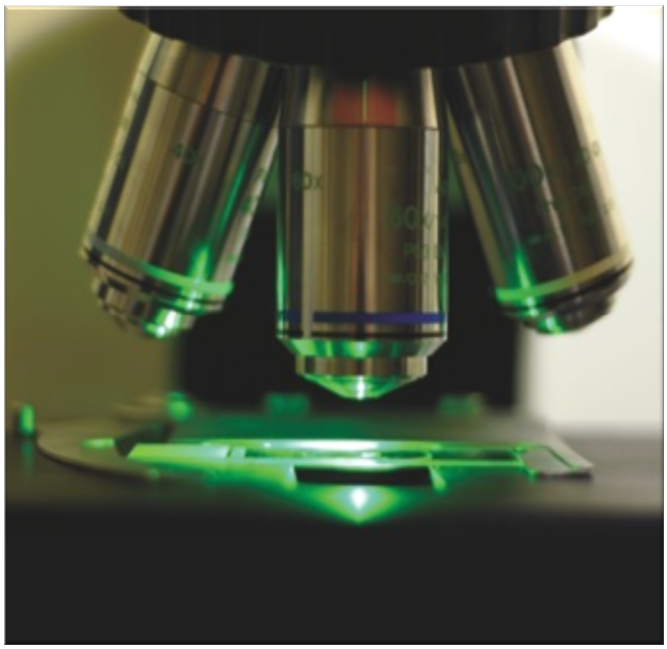
Dr. Rabbi Avram I. Reisner, Rabbi of Congregation Chevrei Tzedek in Baltimore, MD

The Maryland Stem Cell Research Fund (MSCRF): Background, Changes in Federal Policy, Updates, Highlights and Benefits of the MSCRF to the State of Maryland

Margaret Conn Himelfarb, MPH; Chair, Maryland Stem Cell Research Commission (MSCRC)

Stem Cell Research Offers Great Promise to People with Devastating Diseases and Disabilities

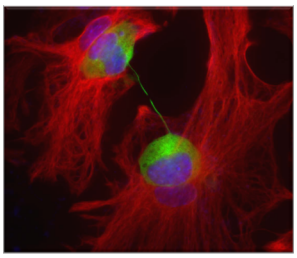
Stem Cell Research helps us understand early human development, including how cells differentiate to become all the organs and tissues in the human body. In addition, stem cells can be useful in drug development and toxicity testing. They can also serve as models of injury and disease. Stem cells have demonstrated their potential to provide cellular therapies and replacement tissues for over 80 diseases, injuries, and conditions, ranging from Alzheimer's, Parkinson's, and Sickle Cell Anemia to diabetes, heart disease, spinal cord injury, and bone repair. Such treatments may ultimately be individually tailored to best match a person's unique needs and genetics. The Maryland Stem Cell Research Fund was created to promote State-funded stem cell research and cures through grants and loans to public and private entities in the State.

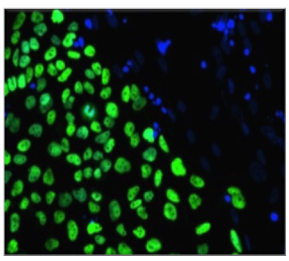


The MSCRF Supports Key Research Projects at Public and Private Entities Throughout Maryland

The Maryland Stem Cell Research Act of 2006 established the Maryland Stem Cell Research Fund, which has received State funding for the past four years, expediting the development of new therapeutics and enhancing Maryland's position in the burgeoning field of biotechnology. This legislation also created an independent Stem Cell Research Commission, which operates under the auspices of the Maryland Technology Development Corporation (TEDCO). The Commission establishes policies and procedures, drafts Requests for Applications (RFAs), ensures the appointment of qualified, independent scientific peer review panels, and administers the Fund, focusing on how to fairly select the most scientifically meritorious ethical research projects.

Impact on biotechnology in Maryland and relevance to regenerative medicine (repair or replacement of damaged, diseased, and defective tissue and organs) are important criteria for all funding awards. Most importantly, the MSCRF has supported a variety of for-profit companies, non-profit institutions and organizations throughout the State. In response to input from several Maryland legislators, the Commission encouraged collaboration between private companies and academic institutions. As a result, the MSCRF now funds 41 collaborative projects, including a dozen partnerships between for-profit Maryland companies and academic institutions. There are also cross campus collaborations between Maryland public and private research institutions.





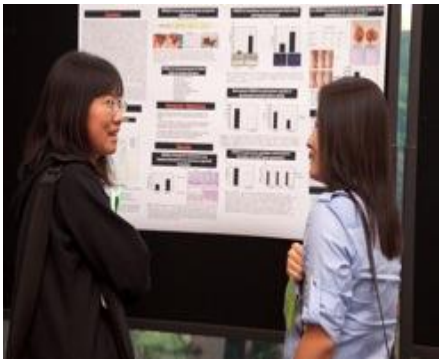
The MSCRF Leverages State Dollars

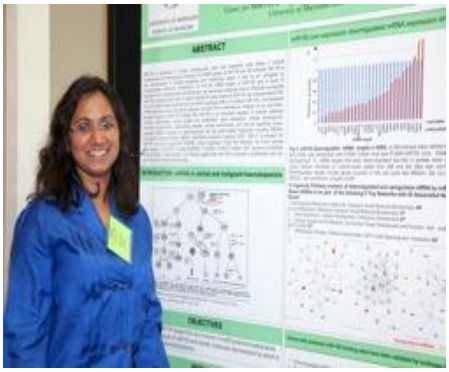
At the 2009 World Stem Cell Summit, Governor O'Malley announced a collaboration between the MSCRF and the California Institute for Regenerative Medicine (CIRM), the nation's first state-to-state, jointly-funded stem cell research program. This collaboration has the potential to create extraordinary research teams and leverage Maryland's financial commitment with up to $76 million from California. The first MSCRF-CIRM grant applications are due in January 2011. In addition, the MSCRF supports several collaborations between in-State Universities and research institutes outside of Maryland, including the University of Pennsylvania, The Scripps Research Institute, and University of Newcastle in England. Because the MSCRF funds only research conducted in Maryland, these partnerships also augment State dollars. Furthermore, Maryland leverages its investment in stem cell research every time an MSCRF-funded investigator receives support from NIH or another resource to advance his work, and whenever a scientist anywhere in the world cites an MSCRF-funded investigator's publication to further his own research.

The MSCRF Shines a National Spotlight on Maryland, Highlighting Its Dominant Position in the Life Sciences

The MSCRF positions Maryland as a center for stem cell research and a role model for the nation. All MSCRF awardees must present their findings at an annual research Symposium, which provides a rare opportunity for investigators from the public and private sectors to share ideas and information. The Third Maryland Stem Cell Research Symposium was held on September 22, 2010 at the National Institute for Standards and Technology (NIST), in collaboration with the National Institutes of Health (NIH), Johns Hopkins University, and the University of Maryland Baltimore. At this event, 20 speakers and 130 poster presentations showcased Maryland's investment in stem cell research to more than 350 participants from academic institutions, biotech companies, State and federal agencies, patient groups, non-profit organizations, and the general public.

The Genetics Policy Institute (GPI) selected Baltimore as the site for its 2009 World Stem Cell Summit. The largest stem cell symposium in the country last year, the Summit was hosted by Johns Hopkins University, the University System of Maryland, the University of Maryland Baltimore, the MSCRF, TEDCO, and the Maryland Department of Business and Economic Development (DBED). It attracted 1,200 participants from 40 states and 27 countries, including scientists and researchers; industry representatives; stem cell research funding organizations and economic development officials; members of Institutional Review Boards and Stem Cell Research Oversight Committees; patients and patient advocacy groups; government agencies like NIH, FDA, and NIST; and faculty and students in medicine, physiology, biology, ethics, and law. Many Commission members played an active role in this Summit, which has become a prototype for other stem cell meetings around the world. MSCRF director Dr. Dan Gincel and two MSCRF Commission members were invited to represent Maryland and speak at the 2010 World Stem Cell Summit.





The MSCRF Operates Efficiently and Funds a Variety Human Stem Cell Research

The Commission typically holds five meetings a year. Three are open to the general public with the exception of the grant review and selection meeting that is held in closed session to protect confidential information of the applicants. Ninety-three cents of every dollar directly funds research. ("Tracking and assessing the rise of state-funded stem cell research"; Nature Biotechnology; Vol. 28; No. 12; December 2010; Page 1247)

The Commission Has Established Three Funding Mechanisms or RFA's

(1) **Investigator-Initiated Research Grants**, which are designed for established investigators who have existing preliminary data to support their grant applications. Investigator-initiated Research Grants currently provide up to $200,000 in direct costs per year, for up to three years;
(2) **Exploratory Research Grants**, which are designed for investigators new to the stem cell field (young investigators and scientists from other fields) and for new hypotheses, approaches, mechanisms, or models. Little or no preliminary data is required to support these applications. Exploratory Research Grants provide up to $100,000 in direct costs per year, for up to two years. They frequently lead to much larger grant awards;
(3) **Postdoctoral Fellowships** (added in FY '08), which are designed to train postdoctoral fellows in human stem cell research in Maryland. Post-doctoral Fellowships provide $55,000 per year, including all direct and indirect costs, for up to two years.

Through these three mechanisms, the MSCRF funds both basic science and translational research projects that use a variety of types of human stem cell types: adult, cord blood, embryonic, induced pluri-potent (iPS), and cancer stem cells. During their funding period, all awardees must submit their interim and final research results in yearly progress reports.

The MSCRF is an Economic Engine for the State, Creating Jobs and Generating New Revenue

MSCRF grants support researchers, physicians, and lab technicians as well as scientists working in labs supported by its research grants. Using data from the first two years of MSCRF funding (FY '07 and '08), the Sage Policy Group conducted an independent economic development impact study, published in February 2010 (see table below), which highlights the fact that the MSCRF has become an important economic engine for the State as well as a mechanism for developing cutting edge research. The Sage study reports that during its first two years of operation the MSCRF funded more than 500 local jobs and generated $71.3 million in business sales.

(The MSCRF has Committed $68.4 Million to 181 Research Projects Over Four Years

Fiscal Year 2007: 85 applications received; **$15 million** committed to fund 24 new projects (7 Investigator-Initiated and 17 Exploratory Research Grants)

Fiscal Year 2008: 122 applications received; **$23 million** committed to fund 58 new projects (11 Investigator-Initiated, 32 Exploratory, and 15 Post-Doctoral Fellowship Research Grants)

Fiscal Year 2009: 147 applications received; **$18 million** committed to fund 59 new projects (6 Investigator-Initiated, 32 Exploratory, and 21 Post-Doctoral Fellowship Research Grants)

Fiscal Year 2010: 141 applications received; **$12.4 million** committed to fund 40 new projects (5 Investigator-Initiated, 19 Exploratory, and 16 Post-Doctoral Fellowship Research Grants)

Fiscal Year 2011: 255 Letters of Intent received; **budget, $10.4 million**; application deadline, January 2011; scientific review, March 2011; Commission review, May 2011; awards announced, June 2011

Economic Impact of Maryland Stem Cell Research Fund Grants, FY '07-'08

Type of Impact	Employment	Labor Income ($millions)	Business Sales ($millions)
Direct	250	$21.8	$35.5
Indirect	104	$5.5	$14.9
Induced	160	$6.8	$20.9
Total	514	$34.1	$71.3

(Sage Policy Group Study; Page 3; http://www.mscrf.org/_media/client/pdf/Sage_stem_cell_program_impacts_Final.pdf

"We shall not cease from exploration and the end of all our exploring will be to arrive where we started and to know the place for the first time."

-Thomas Strearns (T.S.) Eliot 'Little Gidding-

Interest in the MSCRF has Grown Exponentially as Funding has Diminished

The escalating interest in the MSCRF clearly demonstrates the program's credibility as well as increased awareness of the importance of this research. Each successive year, many new scientists apply for MSCRF funding. The number of applications has grown from 85 in FY '07 to over 140 in FY '10. For FY '11, 255 Letters of Intent (LOIs) have been submitted, a record number, which is a 32% increase over last year and a 300% increase over the first year. (While there are typically a few more LOIs than final applications, this year's LOIs do not include the additional applications-anticipated in response to the new collaboration with CIRM, which has generated much enthusiasm.) Unfortunately, however, the MSCRF budget has been radically cut. It is currently 55% less than the 2008 budget of $23 million. With appreciably more applications and less funding than in any previous year, this year's grant review will be a daunting task. Any prospect of further budget cuts raises significant concern about the future viability of our stem cell research program in Maryland.

MSCRF-Funded Scientists Study Crucial Research Questions and Over 35 Diseases and Conditions

Some of the 181 research grants funded to date by the MSCRF study human development, exploring overarching questions focused on topics such as how stem cells differentiate. Other projects compare various types of human stem cells to determine the characteristics of each and how these cell types are alike and different. Still others investigate cutting edge therapeutic technologies, applicable to various medical conditions. However, the majority of MSCRF researchers examine stem cells in the context of specific diseases. State funding has already allowed Maryland scientists to explore the use of stem cells in the following diseases, disorders, injuries and areas of technology and human development:

Psychiatric, Neurological and Neurodegenerative - Schizophrenia; Parkinson's; Alzheimer's; Lou Gehrig's (ALS); Huntington's; Central nervous system disorders; and Spinal cord injury

Heart and Blood - Sickle cell anemia; Heart attack; Stroke; Neonatal stroke; Peripheral arterial disease (PAD); Paroxysmal nocturnal hemoglobinuria (PNH); Vascular disease; and Bone marrow disorders

Autoimmune - Type 1 diabetes and Multiple sclerosis (MS)
Cancer - Breast cancer; Prostate cancer; Brain tumor (Glioblastoma); Leukemia (Multiple, Acute myeloid, and Acute lymphocytic); Ewing's sarcoma; Pancreatic adenocarcinoma; and Neural tumors

Others - Osteoarthritis; Cystic Fibrosis; Muscular Dystrophy; Down syndrome; Gaucher's; Burn wounds; Retinal degeneration; Chronic pancreatitis; and Kidney, Pancreas, Lung, and Liver disease.

Human Development - Genetic development; Molecular biology; Human erythropoiesis; Neural hematopoiesis; and Brain pathology

Therapeutic Technology - Stem cell transplantation; Bioengineered cartilage; Bone regeneration; Peripheral nerve regeneration; Muscle repair; Tissue scaffolds; and Chemo-resistance

"The Governor's continued support of stem cell research through the Maryland Stem Cell Research Fund has already had a substantial impact at Johns Hopkins, leading to such breakthroughs as the generation of pluripotent stem cells from patients with sickle cell disease and from patients with brain disorders," Stephen Desiderio, M.D., Ph.D., director of the Institute of Basic Biomedical Sciences and member of the Hopkins Institute for Cell Engineering, stated in the June 16, 2008 press release announcing Governor O'Malley's BIO 2020 initiative.
http://www.governor.maryland.gov/pressreleases/080616b.asp

The Federal Challenge for Stem Cell Research Provides an Opportunity for Maryland Leadership

President Obama signed an Executive Order on March 9, 2009 repealing the policy that restricted federal funding of embryonic stem cell research to only the Bush "presidential stem cell lines." This Executive Order is now being challenged in Federal court, and will almost certainly continue to be challenged until Congress addresses the Dickey-Wicker Amendment of 1995 (which prohibits federal funding for research involving the destruction of human embryos). Maryland is one of only six states that have taken the leadership role typically assumed by NIH in supporting vital research programs. In fact, Maryland is currently the third largest state-funded of stem cell research, behind only California and New York, a critical position at a time of great need. ("Tracking and assessing the rise of state-funded stem cell research"; Nature Biotechnology; Vol. 28; No. 12; December 2010; Page 1247)

While court decisions cast doubt on the immediate status of federal funding for some types of stem cell research, scientists agree that stem cell technology is the future of modern medicine. According to an August 2010 Research! America poll 70% of Americans favor expanding federal funding for embryonic stem cell research. It is reasonable to assume that, as lifesaving breakthroughs occur, people will want to benefit from these discoveries, public opinion will prevail, and federal funding will continue. When that time comes, thanks to the MSCRF, Maryland's stem cell research community will be positioned to successfully compete for NIH funding, with experience and substantive data to support their grant proposals. http://www.researchamerica.org/stemcell_issue.

In fact, because of Maryland's early support for stem cell research, more researchers remain in Maryland than would otherwise be the case, positioning the State to capture a larger share of federal research dollars going forward. (Sage Policy Group study; page 1; http://www.mscrf.org/_media/client/pdf/Sage_stem_cell_program_impacts_Final.pdf

In support of this claim, the Tech Council of Maryland, in its current Policy Platform, recommends that the State "Maintain stem cell research funding at current levels for FY2012. Policymakers should continue stem cell research funding in Maryland at an appropriate level based on the needs of current stem cell funding beneficiaries… This funding allows uninterrupted research opportunities and will give Maryland a competitive advantage once there is clarity on stem cell research policy at the federal level."
http://www.techcouncilmd.com/advocacy/policy_platform.php

Economic Recovery and the Future of Stem Cell Research in Maryland

The Sage Policy Group Study references Maryland's dominant position across the spectrum in the field of biotechnology. Maryland is a national leader in NIH awards, academic bioscience R&D, venture capital, bioscience graduate degrees awarded, and bioscience employees. (SagePolicy Group Study; Page1; http://www.mscrf.org/_media/client/pdf/Sage_stem_cell_program_impacts_Final.pdf

In June, 2008 Governor O'Malley established his platform as a life science Governor when he introduced his signature 10-year $1.3 billion BIO 2020 Initiative. The largest per capita investment in the biosciences made by any state in the country, it included $20 million per year for stem cell research for the next ten years. This initiative was intended to build upon Maryland's preeminence in the life sciences. The State's incubator network, the Maryland Biotech Center, and the Maryland Stem Cell Research Fund are all critical elements of this inspirational initiative.

Because the MSCRF is now nationally recognized, it helps Maryland attract and retain the best and brightest scientists in the field. However, to maintain the credibility of this program, the MSCRF must sustain a meaningful level of funding. If funding continues to decrease, as it has over the past three years, Maryland will send a message to its scientific community that the State is pulling back on its commitment to stem cell research. The risk, of course, is that the MSCRF will lose its synergy when the core of scientists in stem cell research falls below the "critical mass" required to make the program viable. This is likely to happen because (1) Established investigators will leave for California, New York, or other countries that foster this science; (2) Young investigators with families to feed will be forced to choose other areas of research; and (3) Those who attempt to continue this work in Maryland will become more isolated, with dwindling opportunities for fruitful face-to-face exchange of ideas and information with their colleagues.

In these challenging economic times, jobs are important to the State. Keeping researchers in Maryland keeps their jobs in Maryland as well as any further funding they garner from other sources. Postdoctoral Fellowship Grants create new jobs for PhDs and MDs and heighten interest in the field of stem cell research. Exploratory Research Grants provide the unique opportunity for creative scientists to develop new idea that will merit larger grants from other funders. And Investigator-Initiated Research Grants establish a scientist's career and develop a knowledge base on which other scientists can build. These are high-level, well-paying, educated jobs that keep physicians and clinicians as well as researchers in the State.

Like any macro economic project, results can best be measured over time, and stem cell research is a complex young science. But after only four years of funding, it is realistic to expect that scientific breakthroughs are forthcoming. We must step back and envision the broader picture, avoiding the shortsighted temptation to slash funding and forfeit the momentum the State has already gained from its investment in stem cell research. Reducing the MSCRF budget would have devastating effects from which it would be almost impossible to recover.

Maryland's investment in stem cell research is not only critical to advancing science, but also to moving our State's economy forward. The January, 2010 Sage Policy Report claims that the "largest gains are still to come…Stem cell research stands at the cutting-edge of the New Economy." (Sage Policy Group Study Page 1; http://www.mscrf.org/_media/client/pdf/Sage_stem_cell_program_impacts_Final.pdf)

In conclusion, the Maryland Stem Cell Research Commission respectfully requests that Governor Martin O'Malley maintain level funding ($10.4 million) for the Maryland Stem Cell Research Fund for fiscal year 2012. Anything less than that amount will threaten the integrity of the program, jeopardizing not only it's already evidenced momentum, but also the State's leadership role in stem cell research, its contribution to humanity, and expedited economic recovery for the great State of Maryland.

With the support of the Governor and the Maryland Legislature, Maryland will be well positioned at the forefront of the New Scientific Frontier.

[2010]

Investigator Initiated Research Abstracts

MSCRF
MARYLAND STEM CELL RESEARCH FUND

Curt Civin
University of Maryland, Baltimore
Project Budget: $ 1,149,989.00
~~~~~~

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

Short ‘microRNAs’ bind specific messenger RNAs and enhance their degradation and/or block their translation to proteins. Since a single microRNA can block protein translation of many messenger RNAs, microRNAs can serve as powerful switches to regulate cell differentiation. To extend our long-term studies on regulation of blood cell development (hematopoiesis) and stem cell biology, we recently measured microRNA expression in blood-forming stem cells. Based on the microRNAs and their predicted target messenger RNAs expressed in these stem cells, we proposed that many genes specifying hematopoietic differentiation are expressed by blood-forming stem cells, but held in check by microRNAs. The adult blood stem cell-enriched population that we first studied was actually a complex mixture of rare stem cells and various stages of more mature ‘progenitor’ cells. Therefore, to more precisely understand which microRNAs regulate the development of each type of early blood-forming cell, we next profiled microRNA and messenger RNA expression of highly purified subpopulations of adult blood-forming stem cells. We also showed that certain individual microRNAs actually inhibit adult blood cell development, as our model predicts. We determined several of the proteins whose synthesis is blocked by each of these microRNAs and thereby the molecular mechanisms of their effects.

From our profiling of microRNA expression in stem cell subpopulations, we now have a growing list of microRNAs that are highly expressed in 1 or more types and stages of stem cells. We propose to determine which of these microRNAs control stem cell development and other key stem cell characteristics. Then, we will determine how these microRNAs control cell function by revealing the molecules the microRNAs regulate. We anticipate that these fundamental insights into the novel effects of microRNAs in early blood-forming cell development will provide strategies to increase the quantities of hematopoietic and other types of stem cells that can be produced for research and potential clinical transplantation. Finding a method to expand blood-forming stem cells in the test tube is a long-sought goal of stem cell research and would make bone marrow transplantation and gene therapies safer and more effective, and may provide new molecular targets for the treatment of blood cancers. In addition, our work may serve not only to elucidate the role of microRNAs in development of blood cells, but also to serve as a guide to regulation of other types of stem cells. Therefore, understanding how microRNAs regulate stem cells may reveal general mechanisms of stem cell biology and cancer and have a broad impact on technology development for stem cell therapeutics, which is under active development by Maryland academic institutions and corporations.
~~~~~~

Richard Eckert
University of Maryland, Baltimore
Project Budget: $ 1,146,250.00
~~~~~~

Efficient Derivation of Epidermis-Derived Multipotent Stem Cells using Zero Footprint Transduction Technology

The skin surface (epidermis) is comprised of a large reservoir of readily-accessible cells that can be easily tapped for cell therapy applications. Using the epidermis as a source of cells is an attractive plan because the cells are abundant (the skin is the largest organ of the body) and because the epidermis is readily accessible (it is the surface layer of the skin). We have shown that expression of the stem cell maintenance factor, Oct-4, results in conversion of basal epidermal skin cells to multipotent state and that these cells can then be steered to alternate lineages. If the technology required to convert these cells to the multipotent state can be perfected, then epidermal cells may provide an abundant and accessible substitute for embryonic stem (ES) cells. However, this process is not yet efficient enough for biomedical application. The goal of this project is to develop a highly efficient and safe method for the production of multipotent stem cells and for their conversion to cells, such as neurons, that are optimized for use in cell therapy. This is a joint collaboration between investigators at the University of Maryland School of Medicine Department of Biochemistry, and a major Maryland-based biotechnology company, Life Technologies, Inc. of Frederick Maryland. Businesses of Life Technologies include Applied Biosystems and Invitrogen and brands include Ambion, Dynal, Gateway, GIBCO, Lipofectamine, Molecular Probes, Novex, SuperScript, TaqMan and TOPO. Life Technologies sales were $3 billion in 2008 and the company employs nearly 10,000 people and has a presence in more than 100 countries. Life Technologies was formed as a combination of Applied Biosystems and Invitrogen in 2008. The University of Maryland School of Medicine is a major biomedical research institution in Maryland and has total peer-reviewed federal and foundation grant support in excess of $400 million placing it in the top fifteen research medical schools in the United States. Dr. Eckert's department, Biochemistry and Molecular Biology, also ranks among the top fifteen biochemistry departments nationally. We anticipate that the two Maryland-based institutions, the University of Maryland School of Medicine and Life Technologies, Inc. have the complimentary wherewithal to complete the testing and development of the BacMAM system for the production of iPS cells, and bring this system to the biomedical marketplace. Support for this effort will immediately create new jobs and bring new stem cell expertise and training to the State of Maryland. We anticipate that this creative activity will spur additional Maryland-based job creation in the future.
~~~~~~

Yoon Young Jang
Johns Hopkins University
Project Budget: $ 1,150,000.00
~~~~~~

Developing Safe and Effective Stem Cell Technology for Liver Disease Modeling and Therapy

Human induced pluripotent stem (iPS) cells are generated by reprogramming adult cells such as skin and blood cells back to a stage similar to human embryonic stem (ES) cells. It has been shown that iPS cells can be maintained in cell culture conditions for long periods of time and can be used to produce many specialized cell types, therefore they have been proposed to serve as sources for future cell replacement therapy. In the mean time, the differentiated cells from iPS cells can also be used for screening drugs to develop new drug treatment. However, there are several important questions that need to be addressed before we can use iPS cells efficiently.

1) Most of our knowledge about pluripotent stem cells has been gained through the study of human ES cells, and recently it has been shown that iPS cells are not identical to ES cells. It is therefore important to know how different iPS cells are from ES cells. Since iPS cells can be generated from various tissues as opposed to the single origin (i.e. blastocyst stage of the embryo) of ES cells, it is critical to determine whether the difference in cell origins will affect the iPS cell differentiation potentials.

2) The iPS cells need to be safe. The current protocols using retrovirus mediated gene expression pose significant risks for clinical usage. Safer (non tumor inducing) iPS cells without retrovirus integration are needed.

3) In order to apply human iPS cells to studying liver disease mechanisms and drug discovery, disease modeling systems using patient derived iPS cells need to be established.

4) Our current technology to repair the mutated gene in iPS cells is not efficient and needs to be improved in order to use patient specific iPS cells for gene correction therapy.
We have established a panel of human iPS cells from various origins and we have been able to differentiate them into mature liver cells. To make iPS cells more applicable for modeling and treating liver diseases such as α1-antitrypsin (AAT) deficiency, we propose the following study based on our preliminary results. We will improve the technology to generate iPS cells from different origins without viral integration and compare their ability to differentiate into functional liver cells. We will establish a novel disease modeling system(s) in both cell culture and animals by utilizing liver disease patient derived iPS cells. We will also develop new systems to correct the AAT gene mutation in patient specific iPS cells. The corrected iPS cells will be differentiated to liver cells and their functionality and potential for cell therapy will be assessed using a cell culture system as well as animal transplantation models. This study will help to improve our understanding of basic biology of iPS cells, and it will also develop new technologies that can be applied to study and treat many other liver diseases including Wilson's disease, AAT deficiency, Tyrosinemia, Liver Cirrhosis, and liver Cancer.
~~~~~~

George Ricarute
Johns Hopkins University
Project Budget: $ 818,512.00
~~~~~~

Stem Cell Transplantation in Non-human Primates with Parkinsonism

The present proposal is a translational collaborative project to assess the utility of induced pluripotent stem (iPS) cells to restore dopaminergic innervation in an animal model of Parkinsons Disease (PD). In particular, we seek to determine if iPS can be used to repair and/or promote regeneration of damaged dopamine (DA) neurons in non-human primates, with an eye toward performing similar stem cell transplants in PD patients. The current research will be conducted in squirrel monkeys that are treated with the dopamine neurotoxin, MPTP by uniliateral injection into the carotid artery, thereby causing unilateral parkinsonism. The proposed research will compare and contrast the efficacy of dopamine (DA) neurons derived from two different human induced pluripotent stem (h-iPS) cells as cell therapies in this well established model of parkinsonism . Specifically, the two donor cell types will be tested for their ability to reverse hemi-parkinsonism in squirrel monkeys with unilateral nigrostriatal DA lesions produced by intra-carotid MPTP injection. Each donor cell type will be tested in a group of 8 monkeys. Donor cells will be transplanted into the striatum (putamen) ipsilateral to the MPTP lesion; the contralateral striatum will receive an identical injection of the vehicle. Beginning one week after transplantation, monkeys will be monitored for signs of behavioral recovery (reversal of hemi-parkinsonism). Six months after transplantation, animals will be sacrificed for assessment of stem cell survival and migration, and for quantitative studies of host and donor DA cell innervation, signs of rejection, teratoma formation and genetic alteration. To our knowledge, behavioral functionality of DA neurons derived from h-iPS has yet to be demonstrated in a primate model of PD. Collectively, results from the proposed studies will shed light on the potential of iPS to reverse experimental parkinsonism in non-human primates. Findings in non-human primates will have implications for humans and begin setting the stage for similar cell therapy trials in PD patients.
~~~~~~

Piotr Walczak
Johns Hopkins University
Project Budget: $ 1,150,000.00
~~~~~~

Induced Pluripotent Stem Cell-Derived Oligodendrocytes in Therapy: Advanced Tools for Monitoring Engraftment and Evaluating Therapeutic Effect

Stem cell therapy for brain diseases is currently considered very promising, as functional improvement has been observed in both animal disease models and in patients. With significant progress being made in developing stem cell therapies, it is important to focus on the key features important for treatment of a large population of patients. First, the cells must be accessible in large quantities, sufficient to provide therapy for all patients affected by the disease; and second, the cells must be identical immunologically to the cells of the individual patient in order to avoid the need for toxic treatment that attempts to protect the transplanted cells. Recently, scientists have discovered a method for altering cells from adult humans to cells that are similar to embryonic stem cells.

If the properties of such induced stem cells are truly the same as those of embryonic stem cells, it would allow not only the avoidance of the ethical controversy associated with obtaining human embryonic stem cells, this would provide an unlimited source of stem cells without the need for immunosuppression. In this project, we propose to use non-invasive imaging technologies (MRI and bioluminescence), electrophysiology, and immunohistochemistry to evaluate the potential of human-induced pluripotent stem cells to become functional glia and improve neurological function in myelin disease. Our long-term goal is to establish protocols for the generation of patient-specific glial progenitors that can be used for treatment of myelin diseases such as multiple sclerosis or transverse myelitis.
~~~~~~

[2010]

Exploratory Research Abstracts

MSCRF
MARYLAND STEM CELL RESEARCH FUND

Dmitri Artemov
Johns Hopkins University
Project Budget: $ 229,009.00
~~~~~~

Noninvasive Imaging of Stem Cell Homing and Viability

Human stem cells can be used as a novel and exciting tool for therapy of multiple diseases including injuries and degenerative diseases of CNS, heart, liver, and other organs. One of the key requirements for successful therapy is precise implantation of the cells to the site of injury or successful cell migration to these sites. In recent years several noninvasive imaging modalities have been developed that can visualize spatial distribution of implanted stem cells in the body. Unfortunately it is much more difficult to verify that these cells are still alive when they finally reach the destination or the target lesion. Existing strategies to visualize living vs dead cells require an extensive genetic engineering and there are unanswered questions regarding therapeutic potential and clinical safety of those genetically modified stem cells. In this proposal we will develop a novel MRI marker that will enable simultaneous imaging of stem cell localization and can discriminate between live and dead or dying cells. All experiments will be performed with preclinical mouse models of brain injury. However, as the method uses traditional clinically approved contrast agents we envision its straightforward translation to clinic and its future application in clinical studies and cell based therapy of multiple diseases.
~~~~~~

Peter Calabresi
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Dissecting The Mechanism Of Action Of Human Mesenchymal Stem Cells In Experimental Autoimmune Encephalomyelitis

Multiple sclerosis is a debilitating disease that primarily affects young people. There are currently no cures and all the available treatments have significant limitations in their ability to control the disease and all have a significant level of toxicity associated with them. While much information has become available recently about the use of embryonic stem cells to develop new types of therapies, another type of stem cell derived from the bone marrow of adults, known as mesenchymal stem cells have also emerged as having possibly curative and protective properties in some settings. These cells have a number of logistical and theoretical advantages over embryonic stem cells, which may facilitate the development of new therapies using these cells. Mesenchymal stem cells have been reported to downregulate destructive autoimmune responses in animal models. In addition, MS attacks damage specialized cells in the brain that provide protective coatings for nerves called myelin. In some animal models, MSCs have been shown to help keep these specialized cells from getting damaged, which could help the myelin recovery process for patients. We are proposing to conduct experiments that will determine whether these cells might be useful as a new therapy for patients with MS.
~~~~~~

Yung-Nien Chang
Synaptic Research, LLC
Project Budget: $ 229,880.00
~~~~~~

Genetic Engineering the Clostridium difficile Toxin B (TcdB) as a Delivery Vehicle for Re-Programming Factors to Generate Induced Pluripotent Stem Cells (iPSC)

One of the most remarkable discoveries of our time is the ability of stem cells to treat incurable diseases and secure the promise of new breakthroughs in regenerative medicine. The use of human embryonic stem cells, however, has been faced with many stipulations due to the ethical implications of these cells.

In an effort to avoid these limitations, technologies have been developed recently to generate stem cells from non-embryonic cells by introducing certain genes to "reprogram" the cell to a pluripotent form that can then be differentiated. The one caveat to this application is the severe risk of complications that can arise from the reprogramming genes. Since the genes become integrated within the genome of these induced pluripotent stem cells (iPSC), they may remain active even when administered to patients and, if over-activated, could lead to the development of cancer. In response to this serious obstacle to inducing pluripotency with genes, we have now turned to naturally existing mechanisms to deliver reprogramming proteins to cells rather than genes. For example, certain microbial organisms produce toxic proteins capable of self-delivery to other cells. The protein delivery vehicle contains separate domains, each with specific function to enable the delivery of the toxin domain into cells. By engineering this pre-existing biomolecular machine, we hope to use it to carry other cargos and shuttle them into cells by replacing the toxic domain with reprogramming proteins. Once inside cells, such as a human skin cell, these cargos will function to induce pluripotency without the permanence of gene integration. A set of five separate reprogramming proteins will enter the cell, serve their function, and then eventually be degraded, leaving a viable iPSC that is safe for clinical use. The focus of this exploratory research project is to genetically engineer the toxin from Clostridium difficile to be the most effective protein delivery vehicle for iPSC reprogramming factors. Much of the work will involve creating various atoxic forms of the protein. Within the first year, these fusion proteins will be tested on cells that have already been engineered to assess the efficiency of cargo delivery. In the second year, the most efficient form of the protein delivery vehicle will be used to actually generate iPSC with the help of our collaborator, Dr. Zambidis, at Johns Hopkins University. Finally, we will begin to optimize the dosage and formulation of this protein-based product for researcher and therapeutic use. The goal of this research is to develop as safe and efficient method to generate iPSC for use as therapeutic biologics. By transforming non-embryonic cells into stem cells using a novel protein delivery vehicle, the end product is devoid of genetic contamination and ethical concern. With safety being a priority, these stem cells will be able to treat incurable diseases such as diabetes, Parkinson's, and many types of cancer.
~~~~~~

Ping Gao
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

The Role Of C-Myc In Regulating Mirnas And Metabolic Genes In Ips Cells

Induced pluripotent stem (iPS) cells offer unprecedented potential for disease research, drug screening, toxicology and regenerative medicine since they can be readily derived from patients¡¯ blood or skin cells. Just four factors, Oct4, Sox2, Klf4 and c-Myc, permit conversion of adult fibroblasts to iPS cells. How these factors convert adult cells back to pluripotent stem cells remains poorly understood. c-Myc is a master regulator that integrates cell proliferation with cell metabolism. Its abnormal expression is known to cause human cancers. Due to c-Myc¡¯s cancer-inducing properties, iPS cells have also been attempted without the use of c-Myc, however, the efficiency is severely diminished. Although the low efficiency is largely due to the absence of one major function of c-Myc to enhance cell growth, it is unknown whether additional mechanisms are involved. Therefore, we reason that better understanding of regulatory effectors downstream of c-Myc will provide mechanistic insight and hopefully identify ways of permitting efficient conversion without c-Myc¡¯s cancer-causing properties. Recently, we documented that c-Myc suppresses non-coding small miRNAs miR-23a/b, enhances the expression of mitochondrial glutaminase (GLS), and stimulates glutamine metabolism (Gao et al., Nature. 2009; 458, 762-6) .We found that GLS is essential for c-Myc-driven cell growth in cancer cells which share molecular features with those of pluripotent stem cells. Interestingly, other groups reported that miRNAs miR-23a/b were repressed during the induction of iPS cells from adult fibroblasts (Chin et al., Cell Stem Cell. 2009; 5, 111-23), suggesting that a similar regulatory mechanisms mediated by c-Myc may exist in iPS cells. Hence, we propose here to study the potential role of c-Myc in regulating miRNAs and genes such as miR-23 and GLS that have important relevance to metabolism and proliferation in the induction of iPS cells. In this regard, we set the following goals.

Aim 1: To identify miRNAs and genes regulated by c-Myc during the generation of iPS cells using miRNA array and microarray approaches, with specific focus on those related to cellular metabolism and growth.

Aim 2: To determine the role of c-Myc¡¯s downstream targets such as miR-23, GLS, and other targets indentified from Aim 1 that are potentially important for cellular metabolism and groath in iPS cell generation by using gain-of-function and loss-of-function studies.

Our studies will shed mechanistic insight into the roles that c-Myc may play during different stages of iPS cell generation and will link c-Myc to key downstream metabolic nodes that are potentially involved in growth or pluripotency of iPS cells. We hope that our studies will provide novel approaches that permit efficient generation of iPS cells without c-Myc¡¯s oncogenic properties.
~~~~~~

Yung-Nien Chang
Johns Hopkins University
Project Budget: $ 229,563.00
~~~~~~

The Role Of C-Myc In Regulating Mirnas And Metabolic Genes In Ips Cells

The treatments of bone loss due to congenital defects, cancer, and traumatic injuries areassociated with an economic burden that exceeds $40 billion per year. Current treatment modalities, using autologous tissues harvested from other regions of the body, are limited by the amount of suitable bone available and donor site morbidity. Tissue engineering approaches incorporating the use of multi-potent stem cells are viable alternatives and have the potential to provide customized bone grafts for the repair of massive bone loss. Adipose-derived stem cells (ASCs) are multi-potent and readily available from liposuction aspirates, making them a clinically relevant cell source. ASCs can be utilized in conjunction with biomaterial and bioreactor technologies to engineer patient-specific, vascularized bone grafts, with biologic and mechanical functionality. This would revolutionize the way we currently treat large, non-union bone defects. To achieve this however, it is necessary to understand the mechanistic interactions that would: a) enable ASCs within a single tissue construct to differentiate into endothelial (vessel forming) and osteoblast (bone forming) cells, and b) facilitate the synergistic interactions of these two differentiated cell types so that they can undergo spontaneous functional assembly into stable blood vessels and bone tissue. Information arising out of recent studies suggests that within the body, these processes are coordinated by the influence of oxygen concentration in the cellular environments. The proposal therefore investigates the role of oxygen – a major regulator of stem cell fate – in mediating these processes. We designed two specific aims:

In **Specific Aim1**: Oour goal is to characterize the effect of oxygen concentrations on the ability of the ASC population to differentiate into endothelial and osteoblastic cells in the presence of specific growth factors. Additionally, we will use established microarray technology to gain information regarding the broad changes in ASC gene expression in response to environmental oxygen concentrations. This data will illuminate pathways regulating stem cell fate and be used to modify engineering models of bone ascularization.

In Specific Aim 2: The ability of oxygen to
modulate the interactions between the differentiated cells will be determined using cultivation systems designed to distinguish the role of soluble factors versus direct, contact-mediated communication between cells. The results from both Specific Aims will be used to create protocols for generating vascularized bone grafts from ASCs. Our approach is designed to effectively address the needs of orthopedic regeneration, while providing rigorous, mechanistic insight into the biological responses of ASCs. If successful, the proposed work will have significant economic and technological impacts in the state of Maryland and will help to advance the fields of stem cell biology, tissue engineering and regenerative medicine.
~~~~~~

Ahmet Hoke
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Human Embryonic Stem Cell-Derived Schwann Cells To Enhance Nerve Repair In A Model Of Chronic Denervation

Nerve injuries due tophysical trauma are common and cause significant morbidity andlong--term disability. Despite a)ability of peripheral nerves to regenerate and b) significant advancements in surgical repair of injured nerves, functional recovery after repair of injured human nerves remains suboptimal. The failure of functional recovery after human nerve injury is largely due to the requirement for axons to regenerate over long distances to reinnervate their targets. This failure is most secondary to the relatively slow fixed speed of axonregeneration /elongation (about an inch per month in most mammals) and progressive loss of growth supportive environment of the distal stump. The loss of growth supportive environment in the distal stump over time is due to degeration of Schwann cells, which are the primary support cells in the peripheral nervous system.

In this translational research proposal we will attempt to prevent degeneration of Schwann Cells or repair the loss of Schwann cells by using replacement Schwann cells generated from Human embryonic stem cells. In the past 2 years, there has been a significant advance in methods to generate peripheral nerve cells called Schwann cells from human embryonic stem cells. We hope to translate this advancement into improving functional outcome after peripheral nerve repairs.

Specifically, in the first aim of the proposal we plan to generate Schwann cells from human embryonic stem cells and fully characterize them to make sure they have the properties as a native Schwann cell. In the second aim, we plan to replenish the degerated Schwann cells in chronically denervated nerves with fresh ES cell---derived Schwann cells and ask whether they can support axon regeneration in a rat model of peripheral nerve repair that mimics human condition. In the third aim, we ill ask wether we can use ES cell ---derived Schwann cells as “factories” of growth factors for Schwann cells to prevent the native Schwann cells from degenerating in the first place when they are denervated. We’ll then ask if this approach supports axon regeneration after nerve repair.

Overall, our aim is to enhance nerve regeration using stem cells and test it in an appropriate animal model that actually mimics the chronic denervation changes we see in human nerves.
~~~~~~

Vassilis Koliatsos
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Induced Pluripotent Stem Cells as Models of Alzheimer's Disease

The advent of technologies generating stem cells from general body cells, i.e. without a need to break into the blastocyst, has revolutionized our potential to generate any type of cell from normal individuals and patients alike and to study physiological processes, mechanisms of disease, and new diagnostic or therapeutic tools. Taking cells from the skin of patients with early-onset, genetic forms of Alzheimer's disease (AD) allows us, for the first time, to create human nerve cells vulnerable to AD pathology and, in the future, to create neural circuits that harbor AD properties. Such cells and models add substantially to our ability to study mechanisms of AD and to develop novel cellular and drug therapies or new diagnostics.

In this application, we propose to generate stem cells from the skin of patients who harbor a disease-causing mutation in the PS1 gene, to culture and transform them into neurons in dishes, and then to transplant them into the brain of rodents and study their disease properties. Aim 1 of this project focuses on ways to optimize the generation of stem cells from the skin of AD patients using a variety of techniques and methodologies. Aim 2 utilizes the stem cells obtained through work in Aim 1, coaxes them to mature into neurons in the dish or in the brain of rodents, and studies their disease features, i.e. their tendency to generate amyloid peptides and their hypothesized difficulties in maturating into nerve cells efficiently and completely.

This project is a pioneering effort to generate nerve cells with AD properties and study them in the dish and in the nervous system of experimental animals. This effort lays the groundwork for a new generation of disease models for AD, in which individual nerve cells and groups or networks of nerve cells harboring disease properties can be studied and used for the development of novel diagnostic and therapeutic instruments.
~~~~~~

Tibor Kristian
University of Maryland, Baltimore
Project Budget: $ 230,000.00
~~~~~~

Combinatorial RNAi Strategy for Neural Cell Replacement in the Postischemic Brain

Despite a large number of experimental studies and the progress made in understanding the mechanisms of neuronal death, therapeutic advances in stroke treatment have been limited and several pharmacologically based neuroprotection approaches to treat stroke damage have proven unsatisfactory. One of the major limitations of current approaches in stroke treatment is the inability to replace lost neurons. The use of neural stem cells or stem cell-derived progenitors that can replace the function of dead endogenous neurons is a highly exciting new therapeutic direction for treatment of acute and chronic brain disorders including global and focal ischemia.

While several positive reports indicate that stem cell and stem cell-derived neural progenitor cells have a great potential for treatment of stroke and other neurologic disorders, the extremely low rate of survival of the engrafted cells into the hostile brain environment created following injury remains one of the major factors that limit the efficiency of these transplantation strategies. Since the degree of neuroprotection and the ability of neural stem or progenitor cells to replace lost neurons and glial cells are dependent on the number of cells surviving following transplantation, strategies to enhance the survival of these cells into the injured brain are acutely needed.

Although little is known about the mechanisms involved in the death of these cells in the hostile brain environment following transplantation, recent studies suggest that cell death pathways leading to both apoptotic and necrotic death are concomitantly activated in neural stem cells (NSC) and neural progenitor cells (NPC) transplanted into the brain. Our primary hypothesis is that a new protective strategy that targets key molecular mediators of mitochondria-dependent necrotic and apoptotic death both individually and in combination results in increased survival of hENP both in vitro and in vivo.

The following hypotheses will be tested in the project: 1). Test the hypothesis that a new RNA interference-based strategy can be used to increase the survival of human embryonic stem cell-derived neural progenitors following exposure in vitro to stress paradigms relevant to hostile conditions present in the post-ischemic brain. This approach is based on inhibition of mitochondria-dependent apoptotic and necrotic death pathways individually and in combination. 2). Test the hypothesis that human embryonic stem cell-derived neural progenitors modified through RNA interference to be resistant to apoptotic or necrotic death or to both of these death pathways exhibit increased survival when transplanted in the post-ischemic brain.

The significance of this exploratory research is that it could lead to a new protective approach that could ultimately be translated into a clinical tool to improve the effectiveness of stem cell therapy for brain ischemia/reperfusion victims.
~~~~~~

Min Li
Johns Hopkins University
Project Budget: $ 223,159.00
~~~~~~

Small Organic Chemical Modulation of Human Cardiac Potassium Channels

Drug development requires effective tools to profile and prioritize candidate compounds for further testing. Rapid advances in stem cell biology have begun to afford human cells with different tissue origins and of different genetic backgrounds. Our laboratory has conducted three large-scale high throughput campaigns for targets causal to or associated with cardiac diseases. This application is aimed at developing both tools and procedures to evaluate and prioritize newly identified active compounds using differentiated human cardiomyocytes. The impact to biotechnology in Maryland includes (1) technology development and leadership, (2) complementary and synergistic with other key areas of stem cell technologies; and (3) training of new labor force and technological leaders.
~~~~~~

John McDonald
Hugo W. Moser Research Institute at Kennedy Krieger
Project Budget: $ 230,000.00
~~~~~~

Localized Growth Factor Effects on Myelination by hESC Derived Oligodendrocytes in a Novel Microfluidic Platform

Human embryonic stem cells (hESCs) are capable of growing into any type of cell in the body. Therefore, they may someday be used to repair or replace diseased tissue and even entire organs. Our focus is on using them to regenerate and/or repair nerve tissue in the central nervous system, which could help patients with spinal cord injury to regain feeling and movement, and could help treat diseases such as multiple sclerosis. What these diseases have in common is the loss of a protective layer around CNS nerves that send sensory and movement signals between the brain and the rest of the body. This protective layer is like the insulation around an electric wire. When the protective layer is lost or damaged, the signals can't be sent, like an electrical wire with some insulation missing will "short out". Our experiment will use a unique new microfabricated device to grow CNS neurons in the lab in a special way that will allow us to see if hESCs can be used to make the needed protective layer around the axons of those neurons.

To do this we will first grow very special cells called oligodendrocytes (OLs) from the hESCs. OLs are the cells that make the protective layer around axons, and this process is called myelination. These OLs will then be cultured in the new device where they can interact with the axons that need to have the protective layer. The device is specially made with microscopic grooves to keep out the main cell bodies of the neurons, but allow the axons to grow through into another compartment where the OLs will be grown. In this way we can better examine the process of myelination. This has never been done before with OLs grown from hECS.

In the same way we can also see what effect four different chemicals, called growth factors, have on the myelination. These growth factors are normally produced by the body, and understanding their effect on myelination is important so that later on when OLs are used in the body to treat SCI and MS, doctors can also administer supplemental amounts of the growth factors that are determined to be the most beneficial in promoting myelination. Giving the patient extra amounts of the proper growth factors, along with OLs derived from hESCs, will increase the amount and, probably, the quality of myelination of the damaged axons.

The information obtained in this proposed experiment will further the development of clinical therapies using hESCs that will benefit Johns Hopkins Hospital and Medical School, Kennedy Krieger Institute, and the State of Maryland by helping patients, providing new jobs, and additional revenue from the commercialization of stem cell therapy and the microfluidic co-culture platform device that was developed at Johns Hopkins in Baltimore.
~~~~~~

Alison Moliterno
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Defining The Molecular Basis of Aberrant Stem Cell Function in Primary Myelobrosis

The constant maintenance of adequate numbers of circulating red cells, white cells and platelets requires the continuous production of such cells by primitive stem cells in the bone marrow that have the capacity to create both new blood cells as well as copies of themselves. In a class of blood disorders referred to as myeloproliferative disorders (MPD), there is uncontrolled production of blood cells and eventually excess production of the primitive stem cells themselves due to changes in the DNA of genes which normally regulate cell growth. Within the class of MPD, primary myelofibrosis (PMF) is the most aggressive and malignant of these disorders, with a high rate of leukemic transformation and the shortest lifespan, and no specific effective treatments or cures. Our understanding of the cause of the MPD has been advanced in the last few years by the discovery of mutations in two genes which control cell growth, the JAK2 and MPL genes. However, despite similar gene mutations, patients with PMF have unique and aggressive clinical courses compared to other MPD patients despite similar JAK2 or MPL mutations. Thus additional genetic lesions exist in PMF which explain why their disease is so much worse. The goal of this project first is to use a new technology which can more sensitively define gene abnormalities in PMF patients. Second, utilizing a recently developed technology (iPS) that induces differentiated cells to become pluipotent stem cells, this project will generate stem cells from the blood of patients with PMF such that we can study the behavior of the stem cells derived from patients with PMF, ultimately with a goal to understand why these mutations cause bone marrow failure and leukemia in PMF patients. Results from these studies will advance our knowledge of normal and disease stem cells important in blood cancers. A better understanding of the molecular basis of the aberrant stem cell function in PMF will lead to the discovery of prognostic tools and therapeutic targets for PMF patients and for patients with other relatd blood cancers. This project will have broad applications in that it will support stem cell technologies, will enhance the discovery of genes which cause blood disorders, and therefore will be adaptable to a broad range blood disorders.
~~~~~~

Maria Nurminskaya
University of Maryland, Baltimore
Project Budget: $ 200,000.00
~~~~~~

Regulation of Stem Cell-Based Cartilage Bioengineering by Transglutaminase

Deterioration of cartilage in adults due to trauma or disease is a major health issue in industrialized nations. Despite the progress in orthopedic surgery, repair of the injuries to the articular cartilage of the joints remain a significant clinical challenge due to the limited regenerative potential of this tissue. Taking into consideration the impact of this condition on the aging population, creating cartilage implants in vitro through tissue engineering presents a significant clinical need. Human bone marrow-derived mesenchymal stem cells (hBMSCs) are promising candidates for regenerative cartilage therapy owing to their availability from the bone marrow aspirates, and their high potential to become cartilage-specific cells – chondrocytes. Chondrocytes are surrounded by an extensive extracellular matrix that protects the chondrocytes themselves and the underlying bone tissue from injury due to normal use of the joints. Alterations in the composition of the cartilage matrix lead to eventual loss of tissue structure and mechanical properties. A number of special proteins – growth factors and cytokines – are commonly used in cartilage tissue engineering to induce hBMSCs to become chondrocytes. However, these proteins also induce chondrocytes to rapidly mature and become hypertrophic. As a result, hypertrophic chondrocytes deposit less cartilaginous matrix and make a specialized calcified matrix which is eventually replaced by bone. In the cartilage implants, these changes in the newly-deposited matrix lead to formation of the bone-like structures, rupture, and need for repeated surgical replacements. Thus, the major challenge in the hBMSC-based cartilage tissue engineering is generating the chondrocytes which are capable of depositing proper matrix and at the same time are resistant to hypertrophy. Our laboratory is studying a protein that is produced by differentiating hBMSCs, termed tissue transglutaminase (TG2). We have observed that mesenchymal cells produce increased amounts of TG2 as they turn into chondrocytes. At the same time, we have found that the elevated levels of TG2 attenuate deposition of the cartilaginous matrix and accelerate chondrogenic maturation. We have also identified two candidate molecular mechanisms which may mediate these effects of TG2. We hypothesize that reduction of the TG2 protein levels and/or inhibition of its activity in the hBMSC-derived chondrocytes will promote deposition of the cartilaginous matrix while delaying hypertrophy. The proposed study is set to test this hypothesis. We expect these studies will provide both the scientific background and experimental tools for efficient regenerative cartilage therapy using stem cells.
~~~~~~

Akira Sawa
Johns Hopkins University
Project Budget: $ 200,000.00
~~~~~~

Susceptibility To Metabolic And Oxidative Stress In Neurons In Patients With Schizophrenia

Schizophrenia (SZ) is the most debilitating mental illness. Its incidence, about 1% of the population, is the same as diabetes. However, unlike diabetes and other common chronic diseases, SZ is almost totally disabling and a lifelong disorder. Unfortunately, mechanistic understanding of mental disorders, such as SZ and bipolar disorder, is not well developed. One major limitation that has blocked the progress is the difficulty of accessing relevant tissues/cells for study. Therefore, there is expectation that induced pluripotent stem cells (iPS cells) will be a major advance for understanding of mental disorders.

The program for which the PI serves as director at Johns Hopkins, has systematically collected tissues and cells (blood and skin tissues) from patients with SZ as well as normal controls. By utilizing this collection, we have obtained preliminary but promising data on differences in stress response associated with oxidative stress and metabolic signaling between SZ and control cells. A major obstacle at present is that theses data came from peripheral cells, but not neurons relevant to brains. Thus, in the proposed study, we will confirm and further validate our preliminary observations in iPS cells and iPS cell-derived neurons.

This study can lead to translational potential in several ways. First, once this susceptibility associated with oxidative stress and possibly with metabolic overload is shown in both neurons and peripheral cells, this characteristic can be used for a peripheral biomarker, which also has relevance in neuronal cells. Thus, molecular elements associated with such a susceptibility cascade can be further evaluated in clinical settings as to whether they are trait markers or state markers. Trait markers can be used for diagnosis and are especially useful in early diagnosis to detect patients at initial disease stage or even prodromal stage. State markers are useful in monitoring the conditions, especially those in response to medication, more objectively. Thus, these markers can also be utilized for prognosis and may facilitate a better choice of medications in the clinical settings. Furthermore, once the cellular mechanism of this susceptibility associated with oxidative stress and metabolic signaling is clarified, we will be able to extend the study with brain imaging. Indeed, we have already started analyses of subjects from whom we have established iPS cells, also for brain imaging. By matching cell data with brain imaging characteristics, we will be able to address how cellular susceptibility may underlie functional abnormality in neuronal circuitry of brains. This translational approach is expected to promote biotechnology in Maryland.
~~~~~~

Kara Scheibner
University of Maryland, Baltimore
Project Budget: $ 230,000.00
~~~~~~

MIR-23a Cluster and miR-10a Regulation of Self-Renewal and Survival Pathways in Human Hematopoietic Stem-Progenitors Cells and Acute Leukemia Stem Cells

A significant problem in treating leukemia remains recurrence of the disease, even after success of the initial treatment. One possible reason for this may be due to a small population of cells within the leukemia itself. These cells, called leukemia stem cells or LSCs due to the presence of many shared characteristics with adult hematopoietic (blood) stem cells such as survival and self-renewal, have the ability to re-create the entire population of leukemia cells. LSCs are also highly resistant to drug treatment; these cells stay in a non-dividing, or quiescent, state and thus are not killed by current leukemia therapies, many of which target fast growing, highly dividing cells that make up the bulk of the leukemia. While we search for methods to target LSCs, we are also studying regulation and maintenance of normal adult blood stem-progenitor cells in hopes of being able to expand this population of cells, which are able to reconstitute the entire blood system; as such are invaluable for bone marrow transplantation therapies.

MicroRNAs are short pieces of RNA that bind to messenger RNA and either degrade the transcript or block their translation to proteins. We have recently identified a group of microRNAs present in adult blood stem cell-enriched populations, which regulate proteins critical to maintenance of the blood stem cell population and for development of adult blood cells. Other recent work has identified 3 microRNAs (miR-23a, miR-27a, and miR-24-2), derived from the same transcript, or clustered, as well as an additional non-clustered microRNA, miR-10a, that are highly expressed in the adult blood stem cell-enriched population, but not in acute leukemia cells. We have confirmed that one of these microRNAs, mir-27a, functions as a tumor suppressor, and we predict that the others will be as well. MiR-27a effectively targets bulk leukemia cells by decreasing proliferation and increasing cell death.

We propose that decreased expression of these tumor suppressor microRNAs contributes to de-regulation of self-renewal and survival pathway in LSCs, and that over-expression will target LSCs and reduce their frequency in the leukemia population. We also predict that these microRNAs will make the LSC population more susceptible to drug treatment. Conversely, we predict that high endogenous levels of the miR-23a cluster and miR-10a inhibit the expansion and survival of normal adult blood stem cells, and will investigate if manipulation of their expression will lead to expansion of this population. Success of this proposal may lead to novel molecules for developing leukemia treatment strategies, and reveal mechanisms of stem cell expansion, useful not just in adult blood stem cells, but in other stem cell populations, and for the continuing development of stem cell therapies.
~~~~~~

Sameer Shah
University of Maryland, College Park
Project Budget: $ 229,896.00
~~~~~~

Mechanical Influences On The Identity And Organization Of Peripheral Neurons Differentiated From Hesc-Derived Neural Rosettes

Stem cells offer potential for the treatment of a variety of peripheral neurodegenerative disorders, including motor neuron diseases such as amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), bulbar palsies, progressive muscular atrophy (PMA) and various forms of spinal muscular atrophy (SMA). To validate the utility of stem cell-based treatment strategies for such conditions, motor neurons derived from stem cells must ultimately demonstrate functional connectivity with target muscle fibers. Key checkpoints en route to this final goal are sufficient purity of implantable motor neuron precursors, appropriate protein expression and cell morphology at various stages of differentiation and development, and finally, electrical activity and neuromuscular junction formation.

In addition to chemical guidance cues, recent evidence suggests that the mechanical environment of stem cells plays a key role in their differentiation. In addition, the contractility and load-bearing capabilities of the intracellular cytoskeletal framework, as well as tension imposed upon cells due to motility or extracellular influences, dictate the extension and polarity of growing axons in newly differentiated cells. We will therefore test the global hypothesis that mechanical loads are important regulators of the differentiation, organization, and function of peripheral neurons derived from embryonic stem cells. In particular, we will test the role of tension (stretch) and cytoskeletal stability on cell fate and function.

Successfully addressing our hypotheses will define whether, at what stage of rNSC differentiation, and by what degree intracellular and extracellular mechanical cues influence differentiation and axonal organization. This exploratory research will provide a novel regulatory mechanism that may be integrated into human neuronal stem cell culture and implantation strategies, towards the eventual treatment of motor neuron disease.
~~~~~~

Mark Williams
University of Maryland, Baltimore
Project Budget: $ 230,000.00
~~~~~~

Disease-Specific Induced Pluripotent Stem Cells to Investigate Stem Cell Defects in Down Syndrome

Down Syndrome (DS), which occurs in approximately 1 in 700 births, is the primary cause of mental retardation in the United States. DS patients also suffer from immunologic problems, including autoimmunity and susceptibility to infections. Because DS patients exhibit disorders such as early onset Alzheimer's and altered immune function, it has been suggested that DS is a model for premature aging. One possible common thread that has been proposed to link these observations in DS, is a loss of or abnormal stem cell function. Previous studies in mouse models of DS, including our preliminary data, have suggested that both blood and brain stem cells are impaired, but there is little information on human stem cells in DS. Reprogramming of mature cells from the body into stem cells, so-called induced pluripotent stem cells (iPS), has led to the ability to monitor human stem cell function without using embryonic stem cells. Furthermore, the advent of disease-specific iPS has provided an opportunity to study the effects of disease conditions on stem cell development and function under controlled conditions outside of the body. In the current study, we will study human Down Syndrome-specific iPS cells to determine if there are defects in formation of blood and brain stem cells and development into their progeny. We will grow the iPS in culture and induce them to differentiate towards the blood and brain lineages, which we will follow using approaches such as PCR, fluorescence microscopy, flow cytometry and colony formation. We hypothesize that closer examination of the differentiation and biochemical changes in these cells may reveal novel mechanisms of disease development in DS. One potential mechanism that we hypothesize might affect stem cells in DS is the presence of increased oxidative stress, which has been proposed to cause stem cell aging in other systems. Both human DS patients and DS animal models demonstrate increased oxidative stress and it has been suggested that this contributes to premature aging in DS. In this proposal, we will treat DS-specific iPS with antioxidants or treatments designed to diminish oxidative stress to determine if they enhance stem cell function and if it changes the ability to form different cells in the brain or blood lineages. Thus, in this study, we will define human stem cell defects in DS with the goal of identifying novel mechanisms of disease and therapeutic targets. Furthermore, we will determine if increased oxidative stress is one potential mechanism and a means by which stem cell development can be manipulated using counteractive measures that decrease oxidant levels. These findings will be directly applicable to future experiments in animals and humans testing the utility of iPS stem cell therapy.
~~~~~~

Rong-Ze Yang
Unversity of Maryland, Baltimore
Project Budget: $ 229,994.00
~~~~~~

Towards Generation Of Insulin-Producing Cells In Adipose Tissue By In Situ Reprogramming

Diabetes affects nearly 24 million Americans, or about 8 percent of the U.S. population. It not only imposes a long-term suffering on the patients, but also a burden on the country. The overall cost of diabetes exceeded $174 billion in 2007 in the US. Remarkably, the incidence of diabetes is increasing and it is estimated that the number of Americans with diabetes could nearly double to 44.1 million during the next 25 years. These alarming numbers call for urgent effective prevention or treatment of diabetes.

Type 1 is characterized by absolute deficiency in insulin production due to autoimmune destruction of pancreatic beta-cells. It usually attacks children and young adults. Patients with type 1 diabetes are depended on insulin injection for survive. Although insulin injection is an effective treatment for type 1 diabetes, most patients are unable to maintain their blood glucose in the normal range all the time and are prone to dangerous episodes of hypoglycemia. This led researchers to look for pancreatic islet or beta-cell transplant for the treatment. Pancreatic islet transplantation has been proven to be a cure for Type 1 diabetes, but it suffered from the life-time side effect-prone immunosuppressor treatment and the severely shortage of pancreas donors. Thus, generation of insulin producing cells from patients¡¯ own cells is the focus of cell replacement therapy for Type 1 diabetes and holds a great promise for cure of the disease.

Although, with current technology, it is possible to make self-renewable stem cells from patient's own body cells and turn them into insulin-producing cells in principle, this approach likely takes time in order to work out efficient methods to turn the stem cells into insulin-producing cells and to obtain a pure population of the insulin producing cells. Recent great progress in cell reprogramming, i.e. to turn cells from one type to another type by genetic manipulation, prompts us to propose in this application to reprogram adipose stromal vascular cells into insulin-producing cells for the reasons that the adipose tissue is abundant and the adipose cells can give rise to many types of cells including insulin-producing cells.

We will first clone about 30 genes, known to be important for beta-cell formation, into a viral vector. Next, we will introduce these genes individually or in group into human adipose stromal vascular cells which have been embedded a beta-cell marker, and then culture and induce them into insulin-producing cells to find the most potent combination of the pancreatic genes for generating insulin-producing cells from the adipose stromal cells. A success of this approach will allow us to further conduct a pilot study of directly injecting such pancreatic genes into adipose tissue to turn a fraction of the adipose cells into insulin-producing cells for treatment of diabetes, which may represent a breakthrough in diabetes treatment and will have significant social and economic impact.
~~~~~~

Karen Zeller
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Characterization of Novel Sequence Motifs Important for Stem Cell Biology

Elucidating the core transcriptional network in human embryonic stem cells is crucial to our understanding of pluripotency and self renewal as well as cellular differentiation. To date, most effort has focused on deciphering the regulatory network of transcription factors such as OCT4, NANOG and SOX2 that are known to play important roles in stem cell maintenance with little emphasis on discovery of new factors that are also relevant to stem cell biology. Recently, we have discovered a novel sequence motif that is enriched in human promoters that are bound by stem cell factors SOX2, NANOG and MYC. When found in clusters, these motifs are highly conserved and are often located in the proximal promoters of known genes. Thus it is highly likely that this motif is a functional stem cell relevant promoter element. In this regard, gene set enrichment analysis reveals that there is a significant overlap between the genes that contain this promoter motif repeat and genes that are highly expressed in hematopoietic stem cells. This observation along with the fact that this motif is enriched in promoters that are bound by 3 important stem cell factors, suggests that the protein or proteins binding to this motif might be important for stem cell maintenance. Therefore, we propose to identify these factors with proteomic approaches and map the transcriptional network regulated by these proteins. We also propose to expand our search for novel motifs that may interact with MYC, one of 4 factors sufficient to convert human and mouse adult fibroblasts to a pluripotent stem cell (iPS), and other transcription factors in human ES cells by analyzing our MYC genome-wide binding data as well as published data sets. With the discovery of additional factors essential for stem cell biology, we will not only increase our knowledge of pluripotency and self-renewal, but will enhance our ability to reprogram adult cells and to differentiate these iPS lines down specific lineages thus advancing our ability to treat human-disease.
~~~~~~

Jizhong Zou
Johns Hopkins University
Project Budget: $ 230,000.00
~~~~~~

Targeted Gene correction of x-cgd Mutations in Patient-Specific iPS Cells

Human induced pluripotent stem cells (iPSCs) are established from the adult cells, such as skin or blood cells, by converting them back into an embryonic stem cell (ESC)-like state, and can proliferate indefinitely in culture therefore providing an unlimited cell resource for research and therapy. Similar to human ESCs, iPSCs have the potential to become any cell types including blood cells under specific condition. The generation of patient-specific iPSCs opened up the exciting prospects for the use of iPSCs in regenerative medicine.

The conventional cell replacement therapy using a donor¡¯s stem cells for transplantation that is hampered by shortage of matched donor cells and significant mortality due to graft-versus-host diseases. In contrast, another kind of therapy using patients¡¯ own stem cells to treat various diseases has intrinsic advantages. One of candidate diseases ideal for this therapy is X-linked Chronic Granulomatous Disease (X-CGD), a blood disease due to the mutation in CYBB gene. However, cell therapy for genetic disease requires the correction of genetic defect prior to transplantation. Current clinical approaches use viruses to randomly insert a normal copy of gene in the genome to restore function, which can cause mutations and cancers. Unlike blood stem cells that are difficult to maintain and expand, iPSCs proliferate can proliferate unlimitedly and allows efficient selection of the gene-corrected stem cell population before turning them into transplantable blood cells. Novel technology such as gene targeting enhanced by zinc finger nuclease (ZFN) was recently developed by us and others to generate precise genetic modifications and stable gene expression without random integration or using viruses in human iPSCs and ESCs.

We have successfully established iPSCs by reprogramming adult cells derived from an X-CGD patient. Built upon our expertise with human iPSCs/ESCs culture and genetic modification, we propose the following two strategies of developing novel gene and cell therapy to achieve targeted gene correction of X-CGD mutation using the patient-specific iPSCs. 1) Targeted addition of a CYBB mini-gene at the AAVS1 locus in X-CGD iPSCs. We will use ZFN to stimulate cell¡¯s nature DNA repair machinery to insert a CYBB mini-gene into AAVS1 that is non-pathogenic and dispensable biologically, and allowing stable gene expression and selection. This gene addition strategy could be applicable to all the X-CGD patients regardless of their various mutations. 2) Correction of the X-CGD mutation at the CYBB locus in X-CGD iPSCs. This strategy preserves flanking DNA context including important regulatory sequences, thereby provides ideal gene correction and function restoration.

The knowledge and experiences gained from this project will promote application of novel technology in both basic and translational research. The model strategies developed for X-CGD can be also extended to research and treatment of other diseases.
~~~~~~

Qun Zhou
Johns Hopkins University
Project Budget: $ 200,000.00
~~~~~~

Targeting Cancer Stem Cells, a Novel Therapy for Endocrine Resistant Breast Cancer

Estrogen is synthesized in the ovary or peripheral tissues via aromatization. The importance of estrogen in breast cancer is underscored by the fact that estrogen receptor-mediated signaling pathways contribute to the carcinogenic process. The central role of estrogen in breast tumorigenesis has led to the development of hormone therapies that either block local estrogen production by aromatase inhibitors such as letrozole or block actions of estrogen/estrogen receptor (ER) by antiestrogens like tamoxifen. Although hormone therapies have improved the outcomes in patients with breast cancer, the development of resistance to the treatment had become a major concern. The precise underlying mechanism leading to the development of acquired resistance remains unclear, but breast cancer stem cells may lead to the ability of breast cancer cells to bypass normal endocrine responsiveness. The goal of this application is to understand the potential regulators responsible for self-renewal and differentiation of breast cancer stem cells. In this proposal, we will study how microRNAs (miRNAs) like miRNA-128 are able to regulate the stem cell self renewal factor Bmi-1 expression, thus controlling cancer stem cell functions including cell proliferation, stem cell maintenance, and differentiation. Most importantly, inhibition of Bmi-1 by miRNA-128 or possible pharmacological approaches should deplete Bmi-1 dependent cancer stem cells and ultimately reduce the estrogenic stimulus and overcome tamoxifen resistance. Our studies will provide a rationale and experimental basis for using specific inhibitors of Bmi-1 to suppress breast cancer stem cells and to improve treatment of patients with breast cancer.
~~~~~~

[2010]

Post Doctoral Fellowship Research Abstracts

MSCRF
MARYLAND STEM CELL RESEARCH FUND

Cheng-Hsuan Chiang
Johns Hopkins University
Project Budget: $ 110,000.00

~~~~~~

Characterization of iPS Cell-Derived Neural Progenitor Cells and Neurons from Psychiatric Patients with a DISC1 Mutation

Major mental disorders including schizophrenia affect about 7 percent of Americans and are the leading cause of disability for ages 15-44. However, underlying disease mechanisms are poorly understood, and effective treatments are very limited. DISC1 has been suggested as a susceptibility gene of major mental disorders. A frameshift mutation of DISC1 has been shown to associate with major mental disorders in an American family (pedigree H). Recent studies have shown roles of DISC1 in brain development and adult neurogenesis in rodents. Since DISC1 has been shown to have primate-specific exon and splicing isoforms, whether defects due to disrupted DISC1 function can be seen in patients remains to be investigated.

The induced pluripotent stem (iPS) cell technology offers a unique opportunity to model psychiatric disorders. Since mental disorders are suggested to be developmental illness, neuronal differentiation of iPS cells allows us to study defects in neurodevelopment. Such an in vitro system can also be used for drug screening and development of therapeutics. Furthermore, human stem cells have been shown to develop into functional neurons in rodent brains after transplantation, suggesting the possibility of establishing in vivo models of mental disorders with iPS cells. The in vivo models will be a great complementation to current models using non-human cells and animals. The in vitro and in vivo models established using iPS cells can be transferred to biotechnology industry in Maryland for large scale drug development. Results from mechanistic will also help to advance preclinical studies and clinical trials.

During the past two years, I have generated multiple iPS cell lines from seven human subjects. I have also differentiated human ES and iPS cells into neural progenitor cells (NPCs) and neurons. In addition, widespread neuronal integration has been achieved after transplantation of human ES cells into developing mouse brains. We have obtained fibroblasts from seven members of pedigree H. Those fibroblasts will be reprogrammed into iPS cells.

To establish an in vitro model of psychiatric diseases, iPS cells will be differentiated into NPCs and neurons. Proliferation of patient and control NPCs will be compared. Migration of patient and control neurons will be examined. Maturation and synaptic development of patient and control neurons will also be examined.

To establish an in vivo model of human psychiatric diseases, iPS cell-derived NPCs labeled with eGFP will be transplanted into developing mouse brains. Migration of patient and control NPCs and immature neurons as well as maturation and synaptic development of patient and control neurons in vivo will be examined.
~~~~~~

Kimberly Christian
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Understanding Roles Of Mecp2 In Neural Development Using Patient Specific Ipscs

I propose to derive induced pluripotent stem cells (iPSCs) from patient-specific fibroblasts to identify how developmental dysregulation of neural pathways generates the cognitive and physiological deficits associated with Rett Syndrome. Through in utero transplantation of patient-derived iPSCs, we can generate a murine model of this disease based on the same genetic mutation and intrinsic mosaicism that affects the human population. Abnormal neuronal morphology has previously been observed in both post-mortem analysis of patient brains as well as Mecp2 null mice. However, the specific processes that are critically affected by Mecp2 mutations are unknown. I will use this model to identify developmental abnormalities in neuronal populations that are causally relevant to Rett syndrome symptomatology. My extensive experience with in vivo electrophysiology will provide the basis for a functional analysis of this neural pathology. Together, these data will allow us to evaluate the potential of pharmacological intervention or modified iPSC-based cellular therapy to ameliorate or prevent the morphological deficits underlying this disease.
~~~~~~

Karen David
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

The Hippo Pathway In Neuronal Stem Cell Maintenance And Tumorigenesis

The number of neurons formed and maintained in the brain is critical for proper brain function. Understanding the molecular mechanisms involved in regulating organ size such as that of the brain is of great therapeutic interest, as dysregulation of these processes underlies disorders such as tumorigenesis and neurodegeneration. In this proposal, we investigate the role of the Hippo pathway in regulating organ size in the nervous system. In particular, we are testing the hypothesis that the Hippo pathway acts as a tumor suppressor in the nervous system by directly regulating the self-renewal or differentiation of neuronal stem cells. The Hippo pathway is a tumor suppressor pathway whose core components and role in regulating organ size were first elucidated in Drosophila, and further shown to be conserved in mammals by our laboratory and others. The Hippo pathway in mammals is comprised of tumor suppressors NF2, MST 1/2, Sav, and Lats 1/2 that engage in a kinase cascade to phosphorylate and inactivate the oncogene YAP. Although the role of the Hippo pathway on controlling cell number is well established in organs such as the mammalian liver and intestine, the physiological function of the Hippo pathway in the nervous system remains to be defined. Our initial analysis showed that the Hippo pathway components regulate the proliferation and survival of neurospheres isolated from genetic knockout models for the Hippo pathway. Further characterization of the neuronal stem cells in these genetic mouse models, and genetic manipulation of the Hippo pathway components in human brain tumor stem cells will elucidate the importance of the Hippo pathway in the renewal and homeostasis of neuronal stem cells.
~~~~~~

Yasue Horiuchi
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Alteration In Gene Expression In Neurons Derived From Ips Cells From Patients With Schizophrenia

Genetic predisposition in schizophrenia (SZ) is clear, but multiple factors contribute to its susceptibility. Disturbance of dopaminergic neurotransmission has been well known in SZ, but its molecular mechanism remains to be elucidated. We hypothesize that combinations of multiple genetic factors affect expression of several key factors, which may underlie, at least in part, the pathophysiology of SZ. I propose a study in which I will differentiate induced pluripotent stem cells (iPSCs) into neurospheres, and hopefully enrich dopaminergic neurons, and conduct molecular profiling by microarray. Alteration in gene expression is likely to be affected, at least in part, by genetic variations. Thus, I will take bioinformatic approaches by using the mRNA by SNP Browser and try to address whether a set of genetic variations may influence expression of genes of interest in neurons in association with SZ. In concrete, we have generated more than 10 iPSCs from patients and controls, and use them for the experiments under the following two aims: In Aim 1, by using iPSC-derived neurons (neurosphere culture), we will compare gene expression profile between SZ patients and normal controls. We hypothesize that there may be set of genes whose expression profiles are altered in both neurons and peripheral cells of SZ patients compared to normal controls. To address this question, we will generate neurospheres from iPSCs and conduct microarray that Dr. Sawa's lab are familiar with in studies with peripheral cells. Differentially expressed genes between SZ and controls will be analyzed both at single gene basis as well as at functional group basis. Because Dr. Sawa's lab already has lists of differentially expressed genes in peripheral cells, such as lymphoblasts, from the same subjects used for iPSCs, we will compare the gene expression profiles between iPSC-derived neurons and peripheral cells. We will use the analytical protocols that I am familiar with through my previous publications as well as those the Sawa lab has employed. Through this comparison, we will pin down differentially expressed genes in both neurons and peripheral cells of SZ patients (this provides both the neuronal relevance and the usefulness as peripheral biomarkers). We will further validate differential gene expression between SZ and controls in iPSC-derived neurons by quantitative real-time PCR. We plan to pin down 30 genes for study in Aim 2, in which we will address possible influence of genetic variation on different gene expression profile in SZ. We hypothesize that genetic variations may influence the different gene expression profiles between SZ and controls. To explore possible genetic variations (SNPs) that may influence expression of the 30 genes pinned down in Aim 1, we take bioinformatics approach by using the mRNA by SNP Browser. Then we will compare these SNPs with the SNPs that are associated with schizophrenia in recent Whole Genome Association Studies.
~~~~~~

Xiaosong Huang
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Generation Of Red Blood Cells From Patient-Specific Induced Pluripotent Stem Cells

Red blood cell (RBC) is a somatic cell type highly specialized in delivering oxygen to body tissues via blood flow. RBC transfusion plays a critical role in numerous clinical situations such as treating sickle cell disease (SCD), thalassemia and various forms of anemias. All of these RBCs are derived from donors. Disruption of blood collection and transportation by natural disasters, epidemics and terrorist attacks could severely affect blood supplies that are only sufficient for a few weeks even in USA. The ultimate aim of this proposal is to develop a method to massive produce red blood cells from an unlimited source. Human embryonic stem cells (hESCs) can self-renew indefinitely in culture and have the potential to differentiate into any cell types in human body including RBCs. Recently human somatic cells have been successfully reprogrammed into induced pluripotent stem cells (iPSCs) that exhibit characteristics similar to hES. Patient-specific iPSCs can be generated so that their derivatives such as RBCs are fully immune compatible to those patients. They offer the potential to produce patient-specific RBCs, especially for patients who need chronic transfusions or with a rare blood type with limited choices of blood donors. Built upon the expertise on iPSC derivation, genetic modification and hematopoietic differentiation in Dr. Cheng's lab, my previous experience working with hematopoietic differentiation of hESCs and mouse erythropoiesis and the expertise on erythroid differentiation of hESCs of our collaborator Dr. Shi-Jiang Lu at a biotech company ACT, I propose to develop methods to generate human iPSC-derived RBCs in Dr. Linzhao Cheng's lab at Johns Hopkins University.

Specific Aim 1: Promote the erythroid cell differentiation and expansion from human iPSC-derived hematopoietic progenitor cells.

Specific Aim 2: Promote the terminal maturation of iPSC-derived erythroid precursor cells into enucleated RBCs that express adult hemoglobin.
~~~~~~

Young Jin Lee
University of Maryland
Project Budget: $ 110,000.00
~~~~~~

Evaluating the Role of Prion Protein in Self-renewal and Differentiation of Human Embryonic Stem Cells for Neuronal Regenerative Therapy

In the previous studies, a diverse range of cellular activities has been proposed as the possible biological function of cellular form of the prion protein (PrP^C) including involvement in neuroprotection, cell adhesion, cellular development, differentiation or regeneration. However, the precise cellular function of PrP^C has not yet been defined. Our previous data indicated that treatment of hESCs with recombinant prion protein (rPrP) delayed the spontaneous differentiation of hESCs and helped to maintain their high proliferation activity during spontaneous differentiation. Our current study consists of two specific aims, which are focused on analysis of PrP^C role in self-renewal/differentiation of hESCs and characterization of differentiated cells from PrP^C-silenced hESCs for future clinical application. The first aim will evaluate the cellular role of PrP^C in neuronal differentiation using hESCs. To address this question, we will establish hESC lines, where endogenous PrP^C will be conditionally knock-downed by inducible PrP^C silencing or over-expressed using lentiviral expressing system. The effects of temporal silencing or over-expression of PrP^C will be examined during differentiation of hESCs. The second aim will define the stage of hESCs differentiation at which PrP^C could be silenced without deleterious effects on differentiation/ formation of functional neuronal cells. Inducible PrP^C silencing or over-expressed vectors were constructed and lentiviral particles that have these vectors were produced. Using them, establishment of hESC lines that could be regulated expression of exogenous PrP^C or shRNA against PrP^C is in progress. We propose that PrP^C-silenced neurons could be employed for regenerative cell therapy for treating neurodegenerative maladies caused by infectious prions. In future studies, animal models of neurodegenerative diseases will be used to confirm their functions in vivo. If successful, this study will provide us with new important insight about biological role of PrP^C in human neurogenesis and offer new tools for regenerative therapy for treating prion-related neurodegenerative diseases. This study is supported by 2010 Maryland Stem Cell Commission Grant to YJL (Postdoctoral Fellowship Grant).
~~~~~~

Hua Liu
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Towards Generation and Expansion of Clinical Grade Hepatic Cells from iPS Cells

As human stem cells move closer to clinical application, there is an imperative for standardization of cell culture systems. Therefore further efforts are necessary to establish highly defined culture conditions permitting the derivation, expansion and differentiation of stem cells. The presence of xenogeneic animal products in stem cell culture increases the risk of contamination via animal pathogens, may evoke immunological rejection upon transplantation, and the presence of variable and unidentified factors greatly complicates developmental studies. The cell culture industry is making the shift away from animal-based materials and now starts to offer a range of media and reagents for the growth and differentiation of stem cells as it moves towards clinical applications.

Although the directed differentiation from human induced pluripotent stem cells (iPSCs) have been steadily improved, a more efficient method, ideally free of xenogenic feeder, sera and animal proteins, is necessary to apply this approach to broad research and clinical applications. There is a general lack of defined culture conditions to maintain and expand progenitor cells either from primary sources or from iPSC differentiation. This will greatly hinder the application of iPSC technology in clinical purposes.

Recently we have successfully differentiated human iPS cells into all three stage hepatic cells including definitive endoderm (DE), hepatic progenitors (HP), mature hepatocytes. The current differentiation culture conditions for these cell types however are not designed to selectively expand each cell types. Therefore I planned to determine the most suitable differentiation stage of iPS cells for liver regeneration and also develop xeno-free culture conditions for expanding these transplantable liver cells.

Using our stage specific differentiation protocol, I have started to determine which stage hepatic cells are most capable of liver regeneration. After inducing differentiation of iPSCs into liver cells we transplanted each stage liver cells into highly immunodeficient mice. We are now analyzing some of these human cell transplanted mice to determine whether each stage liver cells derived from human iPSCs are able to engraft, survive, proliferate and function like normal liver cells *in vivo,* using analyses of donor cell engraftment by human liver specific markers and liver function tests for the recipient mice. We are also examining several xeno-free culture conditions for expanding each stage liver cells. The eventual best condition(s) will be used to expand the cells in a larger scale and the *in vivo* activity of the expanded cells will be assessed using the protocol developed.

By these approaches I anticipate developing a safe and efficient culture condition(s) and technologies specifically for hepatic cells derived from human iPSCs.
~~~~~~

IL Minn
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Leukemic Stem Cells and Their Microenvironment

Interaction with the microenvironment (i.e. niche) is essential for the maintenance of stemness of primitive hematopoietic stem cells (HSCs). Thus, understanding this interaction in a cellular and molecular level is critical for the applications of HSCs based therapies. Our knowledge about HSCs niche has been obtained from elegant murine *in vivo* studies that revealed the specialized niche components in bone marrow (BM), however there is a limitation on studying the human HSCs niche due to lack of good *in vivo* experimental tool. Although there have been attempts to create human HSCs xenografts in immunocompromised mice to study microenvironmental regulation of human HSCs, it has been reported that murine BM is not capable of supporting proper differentiation of HSCs into functional progenitors *in vivo*. For the same reason, efforts to identify the human leukemic stem cells (LSCs) niche using murine system may not reveal true human LSCs microenvironment. It is important to investigate the LSC niche because the pathogenesis/relapse of acute myelogenous leukemia is related to LSCs dormancy in the BM and capacity to escape conventional therapies.

We proposed to investigate a preferential location(s) of human LSCs in BM and the interaction between LSCs and specific niche components. We identified and isolated human LSCs from patient's primary samples and human leukemic cell lines based on their phenotypic characteristics and ability to engraft in xenograft animal model. When we examined BM of the mouse model engrafted with human LSCs, we found that the majority of LSCs were tightly attached to a sinusoid endothelium or located in a BM cavity, but not to osteoblasts. To identify the niche components capable of supporting and maintaining human LSCs we developed a combined *ex vivo* and *in vivo* assays; 1) *ex vivo* co-culture of human LSCs with potential niche cells (vascular endothelial cells, mesenchymal stem cells, and osteoblasts) in a medium selectively maintains niche cells not LSCs, 2) *in vivo* engraftment assay where we subsequently inject co-cultured LSCs into immunocompromised mice to score the ability to recapitulate the disease. Our preliminary data showed that LSCs cultured with vascular endothelial cells robustly engrafted to the peripheral blood, BM, liver, kidney, and formed additional peritoneal tumor masses. LSCs cultured with mesenchymal stem cells were mostly found in the peripheral blood and BM. Osteoblasts supported proliferation of LSCs best among tested potential nice cells in ex vivo co-culture but LSCs culture with osteoblasts rarely engrafted. We will continue to develop this combined assay for primary LSCs obtained from patience through collaboration with Quality Biological Inc. (Gaithersburg, MD). We will also keep investigating the mechanisms of how niche cells support LSCs using various interdisciplinary approaches including microfluidics technology to quantify the interaction between the niche and LSCs and to screen the identity of the interaction.
~~~~~~

Emily Potter
Johns Hopkins University
Project Budget: $ 110,000.00

Gliogenesis From Multipotent Precursor Cells; Identification Of Novel Differentiation Factors

There is evidence that neural stem cells can be used to recreate specific cell types for the purpose of treating human neurological disease; however the cues that are required to direct neurospheres into specific lineages remain poorly understood. Recent studies have shown that neurospheres can be differentiated into NG2/PDGFRŬ+ oligodendrocyte progenitor cells (OPCs), but the next step in lineage commitment towards a myelinating oligodendrocyte remains an obstacle to clinical translation. We propose to identify compounds that would hold promise for the treatment of myelin disorders including multiple sclerosis (MS). We have generated appropriate reporter cells for these assays including rodent NG2_DsRed; PLP_eGFP and human precursor cells derived from adult brain biopsy tissue. Failure to remyelinate may be attributed to depleted progenitor reserves, unfavorable changes in the peri-lesion environment, axonal injury or a combination of these factors. If progenitors are depleted during chronic demyelinating disease, then therapies aimed at either augmenting the endogenous progenitor population (NG2/ PDGFRŬ+ OPCs) or introduction of exogenous stem cells may be appropriate. However, if an adequate population of progenitors prevails throughout the course of chronic disease, compounds capable of promoting OPC differentiation into oligodendrocytes could be therapeutic. In either scenario, a drug with the potential to promote differentiation of endogenous/exogenous precursors into oligodendrocytes would be of significant value.

Sivaprakash Ramalingam
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Targeted Addition Of B-Globin Gene At A Pre-Determined Locus In Human Stem Cells

A major challenge in the genetic medicine is to modify the human genome at specific sites. The universal process of gene replacement by homologous recombination (HR), also known as gene targeting, is a very powerful methodology for targeted site-specific gene replacement within the human genome. This will avoid random insertional mutagenesis that is seen with other approaches, but HR is a very inefficient process in human cells (1). Gene targeting occurs only rarely in mammalian cells, only 1 out of every 10^6 treated cells undergo HR (1). To make gene targeting a practical tool, the low frequency of HR needs to be improved significantly. Various studies have shown that creation of a targeted genomic DNA double-stranded break (DSB) greatly enhances the rates of local HR by several orders of magnitude in mammalian cells, by activating the cells own repair machinery. In order to correct a mutation that causes a genetic disorder by HR, custom-designed endonucleases, the molecular scissors that are needed to deliver a targeted genomic DSB at or near the mutation site within the human genome, had to be developed. The design and creation of highly specific designer zinc-finger nucleases (ZFNs) makes this approach possible. ZFNs have become powerful tools for delivering site-specific DSBs at or near the mutation site within the human genome to stimulate local recombination, thereby inducing directed genome modifications in human cells. The designer ZFNs were originally created in Professor Chandrasegaran's lab at the Johns Hopkins School of Public Health. Several studies have successfully shown highly efficient targeted genome modifications in human cells and other organisms by using designer ZFNs (2, 3). Thus, ZFN-mediated gene targeting represents a major advance in the ability scientists and researchers to site-specifically and permanently alter the human genome in cells, not only for various biological and biomedical applications, but also for therapeutic applications in future.

To achieve targeted insertion of a therapeutic gene in human cells, it is essential to choose a chromosomal locus that will serve as a safe-harbor site for the transgene addition. Insertion of the transgene at the targeted chromosomal locus should not result in any adverse effect like insertional mutagenesis. CCR5 is one of the major co-receptors used by HIV-1 virus to infect human cells. It is now well established that people with naturally occurring homozygous mutants containing a 32 base pair deletion (ϙβ32) within the CCR5 coding region are resistant to HIV-1 infection (4). These people are otherwise normal and healthy. It appears that CCR5 may not be an essential gene for the human cells and alternate cellular pathways exist in cells to compensate for the defective CCR5 gene. Thus, the chromosomal CCR5 locus could possibly be an ideal "safe harbor" locus within the human genome for targeted addition of other therapeutic gene expression in human stem cells or iPS cells.

Specific Aim1: The main objective of my post doctoral research over the next two years will be to establish an ZFN-mediated gene targeting to insert the ϐ-globin gene along with requisite transcription factor genes at a pre-determined "safe-harbor" genomic site (in my case the CCR5 gene locus) of the somatic human genome to induce reprogramming of somatic cells to generate iPS cells that also express the corrected ϐ-globin gene.
Specific Aim 2: Study the stemness of the iPS cells and characterize the functional properties of the gene modified iPS cells. To asses their ability to differentiate into hematopoietic cells.

In summary, ZFN-mediated strategies for targeted insertion of ϐ-globin and reprogramming transcription factors at the CCR5 locus in human cells would prevent random insertional mutagenesis and increase the efficiency as well as efficacy of functional complementation of ϐ-globin in iPS cells. If this approach is successful, ZFN-mediated gene targeting would be adopted for routine use to functionally complement other faulty genes of the human genome. Furthermore, this approach is also likely to increase the safety and efficacy of gene therapy. This novel approach, if successful, will likely open new avenues in the area of experimental biology and regenerative medicine.

.

Singh Andirudha
~~~~~~

Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Cyclodextrin-Based Tuning Of Peg Hydrogels For Improved Chondrogenesis Of Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) hold great promise in cartilage tissue engineering. However, to enable translation of stem cell therapies, there is a significant need to generate materials that can modulate and enhance differentiation. Here, we have developed a simple strategy to modify poly(ethylene glycol) (PEG)-based hydrogels, which resulted in significantly improved chondrogenesis of encapsulated MSCs. □-Cyclodextrin (□-CD) was introduced to form inclusion complexes with PEG-diacrylate (PEGDA) in pre-gel solution, followed by photopolymerization to prepare PEGDA-□-CD composite hydrogels. This simple strategy for modifying PEG had a significant effect on cell behavior. In addition, chemically-modified CDs, and CD conjugated with various growth factors and peptides, such as Arginine-Glycine-Aspartic acid (RGD), and their effect on stem cell differentiation are currently under investigations with PEG and other biopolymer-based hydrogels.
~~~~~~

Peng Sun
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Influence Of Microenvironment On Human Stem Cells Maintenance And Differentiation

We are investigating the proliferation and differentiation of human pluripotent stem cells (hPSCs) in hypoxia microenviroment, the normal physiologic condition in embryoid bodies. Enhanced proliferation of human stem cells was observed in lower physiological oxygen (hypoxia)(1,2). Plus, spontaneous differentiation of human pluripotent stem cells (hPSCs) in embryoid bodies occurs in the context of a partially hypoxic microenvironment with gradients of nutrients and oxygen as well as specific cell-cell interaction.(These findings suggest that hypoxic microenvironment plays a role in human stem cells survival and differentiation. We hypothesize that while hypoxia favors the maintenance of pluripotency, stem cells that differentiate down specific lineages will reprogram their metabolic profile favoring oxidative phosphorylation with terminal differentiation. Committed tissue stem cell progenitors, however, are expected to maintain a hypoxic metabolic profile due to the hypoxic niches. However, the properties of the committed stem cell progenitor have not been amenable to characterization because the technologies for their isolation have been lacking. In this proposal, we have developed and will adopt a dual fluorescent protein reporter system, termed HypoxCR (Hypoxia and cell Cycling Reporters), which comprises a hypoxia inducible oxygen-dependent-domain tagged fluorescent protein reporter and a fluorescent reporter fused to hGeminin, which permits the detection of dividing cells which are fluorescent in S and G2M phases of the cell cycle. With the purpose of determining whether we could identify committed progenitors in the hypoxic, cycling fraction of cells, we will use immunohistochemistry and flow cytometry to characterize at least four subpopulations of cell from embryoid bodies: non-hypoxic, cycling; non-hypoxic, non-cycling; hypoxic, cycling; and hypoxic, non-cycling cells. This new strategy, if proved correct, will provide a novel approach to isolating committed progenitors from a heterogeneous mass of cells. These progenitors should provide a source for translational studies aimed toward therapeutic regeneration of damaged tissues.
~~~~~~

Brad Swelstad
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Identification and Isolation of Human Germline Progenitor Stem Cells in the Adult Ovary

Decreased ovarian reserve is one of the leading causes of female infertility around the world, especially in women of advanced reproductive age, premature ovarian failure and those receiving chemotherapy. Development of therapeutic strategies for increasing ovarian reserve has generated considerable interest in recent years [2, 3]. For decades the basic doctrine of reproductive biology maintained that female mammals at birth have a non-renewable source of primordial follicles or oocytes. Recently these beliefs have been challenged by findings of juvenile and adult mouse ovaries with mitotically-active germ cells [5] and by the findings of neonatal mouse female germline stem cells (**FGSCs**) [6]. Specifically, a recent study in Nature Cell Biology reported the isolation of proliferative FGSCs from adult mice. These cells were able to expand in vitro and develop into immature oocytes which in vivo generated offspring [6]. Although the origin of FGSCs is under current debate, growing evidence suggests that FGSCs are more closely related to oogonia than the earlier germ cell progenitors, known as primordial germ cells (**PGCs**). For instance, PGCs under the appropriate culturing conditions have the ability to produce pluripotent stem cells known as embryonic germ cells (**EGCs**). Although FGSCs share similar gene expression profiles to PGCs, FGSCs do not form EGCs in culture suggesting a more differentiated cell-type.

The major germline stem cell surface marker is Mouse Vasa Homolog (MVH). MVH is an RNA helicase from the DEAD box family which is required for regulating translation of mRNAs specifically expressed by oocytes [7]. Highly expressed in both females and male mammals, MVH is a common marker for germ cell identification. Mutations in the helicase domain of MVH cause infertility in both genders. During germ cell development, MVH is expressed in early progenitor primordial germ cells. In females, MVH expression continues throughout germ cell development including metaphase II oocytes. Based on the amino acid sequence for MVH, two transmembrane domains were predicted by Zou et al which lead his laboratory to target MVH as a female germline stem cell surface marker and develop novel cell culture strategies to establish mouse female germline stem cells [6, 7].

Embryonic germ cells have demonstrated the capacity to differentiate into germ cells in vitro. Several studies have demonstrated the generation of oocyte-like structures and post-meiotic male germ cells from human embryonic stem cells [8, 9, 10]. It has been shown that a small number of these cells can differentiate spontaneously or by a growth-factor induced culture such as BMP4, BMP7 and BMP8 into primordial germ cells, the progenitors of sperm and egg [8, 9, 10]. Specific genes that can but be used as markers of differentiation include Deleted in Azospermia-Like (Dazl), which has been shown to be a regulator of germ cell development in a diverse number of species. In a 2009 study by Yu et al, Dazl knock-in embryonic stem cells have been shown to produce both sperm and oocytes using a mouse model [11]. Yu et al also demonstrated markers of female germ cells which are only secreted by oocytes, namely zona pellucid proteins (ZP1, ZP2 and ZP3). In addition to Dazl, other factors also influence germ cell differentiation demonstrated by various methods of cell culture, cell selection and cell lines utilized [12].

Based on background findings and our preliminary data, we hypothesize that a population of proliferating germ cells exist in the human ovary which have the ability to give rise to new oocytes. By creating the ability to isolate these stem cells from adult human ovarian tissue and develop methods for their survival and differentiation in culture, we can potentially in the future provide a means to enhance fertility for women with decreased ovarian reserve or undergoing gonadotoxic treatment that will result in new therapies for fertility preservation.
~~~~~~

Zhexing Wen
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Long-Term In Vivo Imaging Of Dopaminergic Neurons From PD Patient-Derived Ips Cells

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic neurons (DA) in the midbrain. One of the characteristic and early features of PD is the olfaction loss, which is present at the earliest stages of PD in more than 90% of PD patients. The cause of the olfactory dysfunction in PD, however, is poorly understood. Recent studies indicate that mutations (A53T, A30P and E46K) or multiplication of Synuclein gene are linked to familial PD and over expression of wild type or mutant forms of Synuclein leads to the abnormal adult DA neurogenesis in the olfactory bulb (OB) and olfaction deficits in rodents. How aberrant forms of Synuclein could trigger abnormal OB neurogenesis and impairment of olfactory function has not yet been elucidated. Human induced pluripotent stem (iPS) cells provide a unique model system for understanding the pathogenesis of PD. In this proposal, we will generate iPS cells from PD patients with aberrant function of Synuclein and healthy controls, differentiate them into neuronal progenitor cells (NPCs) and transplant them into mouse subventricular zone (SVZ). We will then characterize and compare the development of neurons derived from human NPCs in the adult mouse OB, including axon/dendritic development, synapse formation and degeneration, cell death by using two□photon *in vivo* imaging in living animals. The goal of the current study is to investigate the pathological role of Synuclein in the impairment of adult OB DA neurogenesis and the early olfactory dysfunction of PD with the following two specific aims: 1) to characterize the development and survival of dopaminergic neurons from endogenous neural stem cells and those from transplanted human iPS cells in the adult mouse OB *in vivo*; 2) to determine cell autonomous and/or non□cell autonomous role of aberrant Synuclein on the pathogenesis of DA neurons and OB neurogenesis. This *in vivo* study of the pathogenesis of olfactory dysfunction in PD by using human neuronal cells may provide new insight into the etiology of Parkinson's disease and develop new strategies for early prevention.
~~~~~~

Solomon Yap
University of Maryland
Project Budget: $ 110,000.00
~~~~~~

Development Of CCR5Δ32+/+ Ipscs For Treatment HIV Infection And AIDS

The chemokine receptor type 5 (CCR5), a member of the beta chemokine receptor family of integral membrane proteins, is an essential cellular receptor for HIV infection of T cells and macrophages. A clinically relevant genetic variant of CCR5 is delta 32, which is a naturally occurring knockout deletion variant that introduces a premature stop codon and results in truncation of the protein synthesized. Individuals who are homozygous for the delta 32 allele have a non-functional receptor which effectively restricts HIV entry into immune cells.

The goal of our study is to generate a homozygous CCR5 delta 32 hematopoiesis-competent iPSC (induced pluripotent stem cell) line that may be used in the treatment of patients with HIV infection and AIDS. To achieve this, we have proposed to generate iPSCs from cells which have been isolated from human tissues which harbor the delta 32 allele. We have so far identified three potential sources of delta 32+/+ cells, and have obtained approval from the Institutional Review Board of the University of Maryland School of Medicine for the procurement of these samples. These include 1. placentas, 2. cord blood resulting from the delivery of newborns, and 3. peripheral blood from healthy adults, all of which may be obtained through a simple, painless (for 1 and 2), and non-invasive (for 1 and 2) or minimally invasive (for 3) procedure.

We have began collecting such tissues and genotyping them for the CCR5 gene. A small aliquot of either the placenta or cord blood, as well as buccal swabs from healthy individuals from whom we intend to collect peripheral blood, were used in our genotyping studies. Our primers were designed to amplify either a 300 bp product representing the wild type allele, or a 268 bp product representing the delta 32 allele. To date, we have analyzed a total of 8 placental samples, 32 cord blood samples, and 29 buccal samples from adult individuals for their CCR5 genotype. None of the placental samples, whereas one cord blood sample and 3 buccal samples, contained the delta 32 allele. None of these samples, however, were homozygous for the delta 32 gene. Our DNA sequencing results of the 268 bp PCR product in heterozygotes confirmed that these samples were indeed missing the corresponding 32 bp segment and therefore harbored the gene representative of the delta 32 mutation. We are continually collecting tissues and genotyping them in an effort to identify a sample that is homozygous for the delta 32 allele.

In preparation for the generation of delta 32+/+ iPSCs from either cord or peripheral blood, we have also began the isolation and cultivation of mononuclear cells (MNCs) from umbilical cord blood. The MNC fraction from blood samples was isolated by density gradient centrifugation at 435 g for 30 minutes at room temperature using Ficoll-Paque Premium (density 1.077 g/ml). Following the seeding of MNCs at a density of 1 x 10^6 MNC/cm^2 into Fibronectin-coated plates, we were able to obtain a population of adherent fibroblastoid cells after a period of 7 days. The expansion medium consisted of Dulbecco's modified Eagle's medium-low glucose containing 15% fetal calf serum, and we were able to propagate the cells by replating them in non-coated flasks at a mean density of 3.5 to 5.0 x 10^3 cellls/ cm^2. Our next goal is to generate iPSCs from these cultivated cells.
~~~~~~

Chun Zhong
Johns Hopkins University
Project Budget: $ 110,000.00
~~~~~~

Derivation and Functional Characterization of Neurons Derived from Induced Pluripotent Stem Cells of Huntington's Disease Patients

Huntington's disease (HD) is a common neurodegenerative genetic disorder that affects muscle coordination and even cognitive functions. The disease is caused by extended CAG repeats in a gene called HTT. Human HD patients with HTT mutation lose medium spiny neurons (MSNs) in the striatum and transplant new MSNs will correct this genetic defect. Here we plan to use induced pluripotent stem cells from HD patients to differentiate into neurons, especially the MSNs and physiological characterize their properties. We also plan to characterize the function and property and their changes of the MSNs in vivo during the apoptosis progress. Finally we will test the effect of astrocytes to neurons in HD disease with different HTT background.
~~~~~~

Maryland Stem Cell Commission

Maryland Technology Development Corporation (TEDCO)
5565 Sterrett Place · Suite 214 · Columbia Maryland · 21044
(410) 740-9442 Office · www.mscrf.org