MARYLAND STEM CELL RESEARCH COMMISSION (MSCRC)

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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 (TEDCO)

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

Norma Andrews, Professor and Chair of the Department of Cell Biology and Molecular Genetics at the University of Maryland College Park.

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)

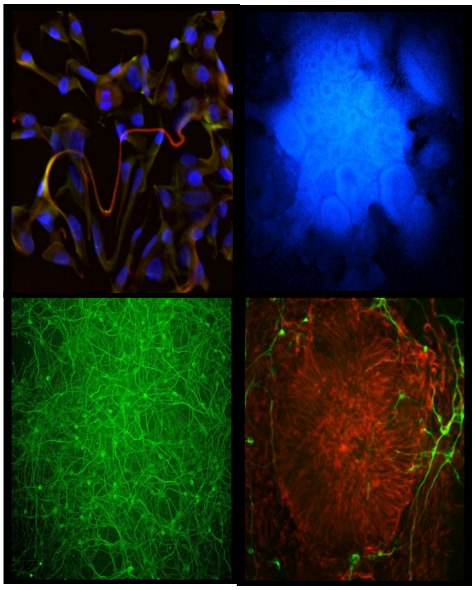
Dr. Jeremy Sugarman, Harvey M. Meyerhoff Professor of Bioethics and Medicine, Berman Institute of BioethicsÂ and Department of Medicine Johns Hopkins University (appointed by JHU)

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

MARYLAND STEM CELL RESEARCH FUND (MSCRF)



STEM CELL RESEARCH OFFERS GREAT PROMISE TO PEOPLE WITH DEVASTATING DISEASES AND DISABILITIES

Stem cells have demonstrated the potential to replace damaged or missing tissues and provide therapies for a multitude of diseases, injuries, and conditions, ranging from Alzheimer's, ALS, Parkinson's, and sickle cell anemia to diabetes, heart disease, arthritis, severe burns, and spinal cord and bone injuries.

Studying stem cells helps scientists understand how cells differentiate into the many types of specialized cells that function in our bodies. Some of the most serious medical conditions, such as cancer and birth defects, are caused by errors that occur somewhere in this process or, in the case of degenerative diseases, later in life. Knowing how normal cells develop will allow us to find treatments that will correct such errors. Patient-derived stem cell therapies may some day be individually tailored to best match a person's unique needs and genetics.

Stem cells are also useful in testing the toxicity of new drugs. In addition, stem cells can serve as models of specific injuries and diseases.

THE MARYLAND STEM CELL RESEARCH ACT OF 2006 WAS ESTABLISHED THE MARYLAND STEM CELL RESEARCH FUND TO PROMOTE STATE FUNDED STEM CELL RESEARCH AND CURES THROUGH GRANTS AND LOANS TO PUBLIC AND PRIVATE ENTITIES IN MARYLAND.

For the past-five-years, Governor Martin O'Malley's annual budget has included appropriations to support the MSCRF. During this period, the Commission has funded 218 research grants with awards totaling over $78.8 million. The FY 2012 annual budget provides $12.4 million for stem cell research in Maryland. This funding serves to expedite the development of critical new therapies, creating new jobs and revenue for the State, at the same time enhancing Maryland's national position in the burgeoning field of biotechnology.

The Stem Cell Research Act also established the Maryland Stem Cell Research Commission (Commission).

The Commission is an independent public body that operates under the auspices of the Maryland Technology Development Corporation (TEDCO).

The Commission establishes policies and procedures, drafts Requests for Applications (RFAs), solicits grant proposals, and ensures the appointment of qualified, independent scientific peer review panels, selecting only the most scientifically meritorious, ethical research projects. The impact on biotechnology in Maryland and relevance to regenerative medicine (repair or replacement of damaged, diseased, and defective cells, tissue, and organs) are important criteria for all funding awards.

The full Commission meets at least four times a year. All Commission meetings are open to the general public except the grant review, which is closed to protect the applicants' intellectual property. 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)

MARYLAND STEM CELL RESEARCH FUND (MSCRF)

THE MSCRF LEVERAGES STATE DOLLARS

At the 2009 World Stem Cell Summit convened in Baltimore, 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. The goal of this collaboration is to advance critical human stem cell therapies by fostering meritorious interdisciplinary research across geographic boundaries. This pilot program has the potential to create extraordinary research teams and leverage Maryland's financial commitment with up to $76 million from California.

The Commission has announced that the collaboration with CIRM will continue for a second year. In FY 2012, the program will allow investigators working in Maryland to conduct collaborative research relating to CIRM's Basic Biology III Grant Awards.

Because the MSCRF funds only research conducted in Maryland, these partnerships 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. continues in FY2012.

THE COMMISSION OPERATES EFFICIENTLY AND FUNDS A VARIETY HUMAN STEM CELL RESEARCH.

The Commission has established several funding mechanisms or RFAs:

(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. (The Commission has revised the grant size and duration of this RFA since the inception of the program.)

(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-Fellowship Grants (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.

(4) In addition to these funding mechanisms, the Commission looks forward to more applications from the private sector in response to its **New pre--clinical and clinical RFA**, which is only for biotech companies interested in conducting late stage human stem cell research in Maryland. This RFA provides up to $750,000 for projects that involve clinical trials, and is currently the Fund's largest award.

Through these four mechanisms and the CIRM pilot program, the MSCRF funds both basic science and translational research projects that use a variety of human stem cell types: adult, cord blood, embryonic, induced pluripotent (iPS), and cancer stem cells.

THE COMMISSION ENCOURAGES COLLABORATIONS TO CREATE THE BEST INTERDISCIPLINARY TEAM FOR EACH PROJECT

In FY 2011, 28% of MSCRF awards involved collaborations between private companies and public institutions, in some instances, across state lines and national borders.

In addition, the Commission continues its second year of a pilot program collaborating with scientists funded by the California Institute for Regenerative Medicine (CIRM). Most importantly, in January 2012, the Commission announced a new RFA directed solely to biotech companies.

THE MSCRF HAS COMMITTED $78.8 MILLION TO 218 RESEARCH PROJECTS OVER THE PAST FIVE 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**: 180 applications received; $10.4 million committed to fund 36 new projects (9 Investigator-Initiated, 13 Exploratory, and 14 Post-Doctoral Fellowship Research Grants)
- **Fiscal Year 2012**: 266 Letters of Intent (LOIs) received from interested applicants; $12.4 million budgeted; scientific review, March 2012; Commission review, May 2012; awards announced, June 2012

INTEREST IN THE MSCRF CONTINUES TO ESCALATE

This increased interest clearly demonstrates the credibility of the program as well as growing awareness of the importance of stem cell research. Each successive year, many new scientists apply for MSCRF funding. FY 2011 was yet another record-breaking year for the MSCRF, with the highest number of applications ever submitted and, for FY 2012, the largest number of Letters of Intent ever received. Any prospect of a budget cut would raise significant concerns about the future viability of our stem cell research program in Maryland.

THE MSCRF IS PART OF A STEM CELL RESEARCH HUB, SERVING AS A ROLE MODEL FOR THE COUNTRY AND ENHANCING MARYLAND'S NATIONAL POSITION IN THE LIFE SCIENCES.

The State's topnotch infrastructure, combined with its highly trained scientists, has engaged and/or attracted a number of leading companies to the area:

• **Lonza** (Walkersville) is the world's largest cell enrichment and clinical manufacturing company

• **Osiris Therapeutics, Inc.** (Columbia) is the world's largest and most advanced stem cell company

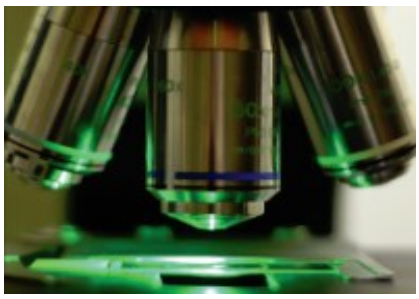
• **Becton, Dickinson and Company** (Sparks) has a large division that focuses on bio-processing and diagnostics, among other interests, with approximately 1,500 employees based in Maryland.

• **Life technologies** (Fredrick) has moved its stem cell R&D from California, and now employs over 500 people in Maryland.

• **The NIH** has created a new intra mural stem cell center and is looking for collaborations with Maryland companies to help develop products.

Maryland hospitals have become leaders in stem cell transplantation, with the largest programs at **Johns Hopkins** and the **Medical Center at the University of Maryland, Baltimore**. **Mercy Hospital** is collaborating to establish the first in-State umbilical cord blood bank for stem cell therapies. **Sinai Hospital** is expanding its stem cell capabilities in research and clinical application.

Included among the new stem cell research organizations are the **Lieber Institute for Brain Development**, a private company located in the John Hopkins Biopark, and **Stevenson University**, which may be the first college in Maryland to train undergraduate students in stem cell research. Many additional new small Biotech companies are eligible to receive funding from the MSCRF.



THE MSCRF IS AN ECONOMIC ENGINE FOR THE STATE, CREATING JOBS AND GENERATING NEW REVENUE.

MSCRF awards 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, 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 only its first two years of operation, the MSCRF funded more than 500 local jobs and generated $71.3 million in business sales. These employment figures significantly increased during the next three years. Furthermore, the Sage Policy Group study predicts that "as research increasingly becomes commercialized, impacts are expected to expand briskly, with economic and fiscal impacts potentially doubling every three years." (Sage Policy Group Study; Page 3; http://www.mscrf.org/_media/client/pd Sage_stem_cell_program_impacts_Final.pdf)

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.

During their funding period, all awardees must submit their interim and final research results in yearly progress reports. All State-funded investigators must cite the MSCRF and credit Maryland in their publications. The fourth Maryland Stem Cell Research Symposium was held on October 5, 2011 at Towson University, in collaboration with Baltimore County. At this event, over 20 speakers and 100 poster presentations showcased Maryland's investment in stem cell research to more than 400 participants from academic institutions, biotech companies, State and federal agencies, patient groups, non-profit organizations, and the general public. The Commission is now planning the fifth Annual Research Symposium, which will be held in Annapolis in the fall of 2012.

ALTHOUGH THE PURPOSE OF THIS ANNUAL REPORT IS TO SUMMARIZE NEW MSCRF-FUNDED RESEARCH, THE FOLLOWING BULLETS HIGHLIGHT SEVERAL EXCITING STATE-SUPPORTED RESEARCH BREAKTHROUGHS THAT OCCURRED IN FY 2011:

- Dr. Sharon Gerecht's lab at Johns Hopkins University developed a Hydrogel that promotes the growth of new blood vessels and scar-free skin, which could be of critical importance in severe burns. (http://www.eurekalert.org/pub_releases/2011-12/jhuitb121311.php)

- Dr. Min Li, Professor of Neuroscience and Director of the Johns Hopkins Ion Channel Center (NIH-MLPCN), was featured on the cover of the journal Assay and Drug Development Technologies for developing a new high throughput screening technology for cardiac drugs using stem cells. Dr. Li is making this database available online at http://www.hergcentral.org/, a new Web site that allows better sharing of results.

- Dr. George Ricarute of Johns Hopkins University, is transplanting stem cells into non-human primates with Parkinson's-type symptoms. This is the last stage of research before human subjects will be allowed to participate in a clinical trial of this treatment.

THE FEDERAL CHALLENGE FOR STEM CELL RESEARCH PROVIDES A CRITICAL 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 still being challenged in federal court and will almost certainly continue to be challenged until Congress addresses the Dickey-Wicker Amendment of 1995 (which is part of the annual budget bill and prohibits federal funding for research involving the destruction of human embryos). A negative decision in that case (Sherley vs. Sibelius) would halt federal funding indefinitely. A change in administration could affect policy and stop federal funding of embryonic stem cell research for a minimum of four years. Furthermore, NIH funding for research in general has declined over the last several years.

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-funder of stem cell research, behind only California and New York, a critical leadership 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...*(http://ww.researchamerica.org/stemcell_issue),* 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.

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)*

The Tech Council of Maryland, in its recent Policy Platform, recommended 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

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. *(Sage Policy Group Study; Page 1; 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 capital 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 relocate to California, New York, or other places that foster this science;

2) Young investigators with families to feed will be forced to choose other areas of research;

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.

FY 2013 BUDGET JUSTIFICATION

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 ideas 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 five years of funding, we are beginning to see scientific breakthroughs like those described above. We must avoid the shortsighted temptation to slash funding and forfeit the momentum the State has already gained from its investment in stem cell research.

The MSCRF FY 2012 budget is only half what it was in 2008. Reducing it more 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 states 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)

Jay Hancock, Economic reporter for the Baltimore Sun, shared that opinion on his blog January 14, 2010 when he wrote: "Biotech is Maryland's one hope for a big, private-sectors urge in employment and investment over the next decade. Stem cell money is the seed for the surge. The General Assembly shouldn't cut the water and fertilizer just when it's ready to sprout."
(http://weblogs.baltimoresun.com/business/hancock/blog/2010/01/dont_miss_biotech_boom_by_cutt.html)

WITH THE SUPPORT OF THE GOVERNOR AND THE MARYLAND LEGISLATURE, MARYLAND WILL BE WELL POSITIONED AT THE FOREFRONT OF THE NEW SCIENTIFIC FRONTIER, WHILE DELIVERING NEW JOBS, AND PROVIDING IMPROVED QUALITY OF LIFE AND INCREASED LONGEVITY TO ITS CITIZENS.

RESPECTFULLY SUBMITTED BY:

MARGARET CONN HIMELFARB , CHAIR
MARYLAND STEM CELL RESEARCH COMMISSION

INVESTIGATOR INITIATED RESEARCH ABSTRACTS

~FY2011~

MSCRF 2011 ANNUAL REPORT

Stem Cell Recruitment in Anabolic Versus Antiresorptive Osteoporosis Therapy

Principle Investigator: **Xu Cao**
Institution: Johns Hopkins University
Project Budget : $690, 000

As our population ages, osteoporosis is a mounting public health problem. Osteoporosis occurs when the amount of bone is so reduced that it no longer maintains it architectural integrity and is prone to fracture. Bone fractures lead to loss of independence, disability and death. Bone is constantly being broken down, or resorbed, by osteoclasts and then rebuilt at the same site by osteoblasts. When osteoclast and osteoblast activity are imbalanced, osteoporosis occurs. The most common medications used to treat osteoporosis arrest bone resorption by inhibiting osteoclasts. Alendronate (ALN) is one such medication. Parathyroid hormone (PTH) is the only osteoporosis medication that builds bone by stimulating osteoblasts. In current medical practice, anti-resorptive agents are used first. Only when there is treatment failure are anabolic agents, such as PTH, prescribed. Evidence in mice suggests that our current practice of using ALN first may not be the most effective way to treat osteoporosis. To better understand the actions of ALN and PTH, it is important to consider the skeleton's mesenchymal stem cells (MSCs). MSCs can mature into several cell types including bone-building osteoblasts. We have shown that when osteoclasts break down bone, growth factors are released. These molecules recruit the MSCs to areas of bone resorption. On arrival, the MSCs become bone-building osteoblasts that will replace the missing bone. In mouse studies, ALN treatment decreased the amount of growth factors released from bone and the number of MSCs recruited to the resorption site. In contrast, PTH treatment stimulated the maturation of MSCs into osteoblasts. When treated with both ALN and PTH, the decrease in growth factors and MSCs caused by ALN also decreased the ability of PTH to build bone. The effect of ALN versus PTH on MSCs in humans and the role of growth factors in balancing the effects of ALN and PTH on MSC recruitment and transition into osteoblasts is unknown. To answer these questions, we propose a clinical trial in three groups of post-menopausal women with low bone density. Women will be treated for 3 months with ALN, PTH or calcium and vitamin D. Blood, bone and bone marrow samples will be collected and analyzed. We think that in humans, PTH will increase MSC number in bone, increase MSC recruitment to bone resorption sites, and increase MSC number in the bloodstream and that ALN will have the opposite effect. To investigate our hypothesis, we will: Investigate the effects of PTH versus ALN on bone formation, structure and presence MSCs; measure the effects of PTH versus ALN on MSC transformation into osteoblasts; quantify the effects of PTH versus ALN on MSCs in the bloodstream. Understanding the role of the MSCs in bone formation with PTH and ALN treatment in humans is of critical importance and can be immediately translated into medical practice to direct more rational and effective use of PTH and ALN in osteoporosis treatment.

Red Blood Cell Production from Human iPS Cells of Transfusion Dependent Patients

Principle Investigator: **Linzhao Cheng**
Institution: Johns Hopkins University
In collaboration with The California Institute for Regenerative Medicine (CIRM) & MaxCyte Inc.
Project Budget : $690, 000

Blood transfusion is one of the most common cell therapies. Red blood cells (RBCs) are a main component of blood, and are responsible for carrying oxygen throughout the body. People with diseases such as severe aplastic anemia, thalassemia major, and sickle cell disease lack properly functioning RBCs, leading to severe medical problems. Such patients require frequent transfusion of donor-matched cells for symptom abatement. However, chronic RBC transfusion can lead to a strong immune system response, causing the patients to reject new transfused RBCs. For this reason, it is necessary to develop methods to produce patient-matched RBCs that are safe for clinical transfusion. To accomplish this goal, we plan to utilize induced pluripotent stem (iPS) cells, which are derived by using molecular biology techniques from adult tissues, such as blood and skin. These cells are similar in function to human embryonic stem cells; they can proliferate indefinitely in the laboratory, but still retain their ability to generate any other cell type, including RBCs. Because iPS cells are developed directly from the patient, immune responses and rejection of transplanted cells could be avoided. For this project, the novel iPS cell lines will be derived from transfusion-dependent patients suffering from aplastic anemia and sickle cell disease. The novel iPS cells will be derived and expanded under clinically applicable conditions. Once enough iPS cells are collected, we will allow them to form RBCs in the laboratory. This study will let us develop methods for mass production of patient-matched RBCs that would be safer for clinical transfusion and have no risk of rejection. Laboratory manufactured RBCs that have a longer shelf-life and less contamination of infectious reagents will allow for more effective treatment of these transfusion-dependent patients and others who need transfusion of their own type or better matched RBCs.

Direct Generation of Human Dopaminergic Neurons By Defined Factors

Principle Investigator: **Mihoko Kai**
Institution: Johns Hopkins University
Project Budget : $690, 000

Scientists are able to generate embryonic stem (ES) cell-like cells, called induced pluripotent stem cells (iPSCs), from human skin, blood and other cells. Like ES cells, iPSCs can be differentiated into many cell types, but unlike ES cells, these cells do not present ethical issues and problems of immunorejection. Although additional research is needed, iPSCs are useful tools for drug development and modeling diseases. Scientists also hope to use them in transplantation medicine. However, the processes of generating iPSCs are time consuming and expensive. Our group has succeeded to generate induced human neurons directly from skin and other cells efficiently. In this proposal, we aim to generate specific types of induced human neurons from skin, blood and other cells, focusing on dopaminergic and glutamatergic neurons. These cells will be useful for developing efficient and safe therapeutic tools to cure diseases such as addiction, depression, ADHD (attention deficit hyperactivity disorder), schizophrenia and Parkinson's disease. In addition, the induced neuronal cells will provide new tools to understand human neuronal development.

Stem Cell Therapies In Animal Models of Traumatic Axonal Injury

Principle Investigator: **Vassilis Koliatsos**
Institution: Johns Hopkins University
In collaboration with The California Institute for Regenerative Medicine (CIRM)
Project Budget : $690, 000

Many types of traumatic brain injury, for example after motor vehicle accidents or in military combat, involve widespread damage to axons, i.e. cell specializations used by neurons to communicate with each other, of great importance particularly to higher nervous system functions as well as functions related to balance and gait. These devastating pathologies are called diffuse axonal injury or traumatic axonal injury (TAI). TAI is associated with high mortality in the acute phase and with severe as well as chronic neurological and psychiatric disability in survivors and does not have satisfactory treatment at the present time. In this application, we propose a cellular therapy for this problem, based on our enthusiasm from previous outcomes of research in our laboratory, where we showed that human stem cells committed to making cells for the nervous system (neural progenitors or NPs) can become fully differentiated nerve cells that then begin to engage in synaptic communication with other nerve cells in the brain and spinal cord. We hope that NPs that make nerve cells will replace damaged nerve cells and axons. We also expect that NPs that grow into oligodendrocytes, i.e. cells that wrap themselves around axons and facilitate conduction of nerve signals among other things, will add to the restorative effect of nerve cell-making NPs. We test these ideas in a rat model of TAI that has been very well characterized, i.e. the impact acceleration model of Marmarou. In the first part of our application, we generate the NPs we need to produce nerve cells or oligodendrocytes after we transplant them into the brain and make sure that, after transplantation, these NPs survive and truly become the cells of the desired fate. In the second part, we wait longer after transplantation in order to make sure that nerve cells and oligodendrocytes made from the NP transplants help form new circuits in the brain and, importantly, that these circuits can help restore critical functions in our experimental subjects, i.e. improve various types of memory as well as motor and sensory functions. Based on our previous experience in other animal models and our partnership with traumatic brain injury and other stem cell experts we hope that we will achieve our goal of at least partial restoration of brain circuits and functions in injured animals with human stem cells. The use of human stem cells as sources for transplants and the inclusion, in our stem cell armamentarium, of clinical-grade cells makes this project very applicable to future clinical trials for patients with traumatic brain injury.

Single Cell Microencapsulation For Ischemic Heart Disease Therapy

Principle Investigator: **Dara Kraitchman**
Institution: Johns Hopkins University
Project Budget : $689,836

Methods to treat heart disease have improved over the last several decades and many more patients survive a heart attack. However, almost 13% of heart attack patients die within a year and almost 1/3 of heart attack patients experience another heart attack or heart failure. Many clinical trials have been performed to study the safety and effectiveness of stem cells as therapy to repair damaged hearts or lessen the likelihood that a person will develop a failing heart. But stem cell therapies are hampered by the fact that close to 95% of the stem cells die within 1 day to 1 week after they are delivered. As a result, it is not surprising that these trials have shown poor results in patients.

Our group has developed a novel technique that can provide a protective bubble around individual stem cells to enhance their survival. This bubble also incorporates agents that allow us to see the stem cells in the bubble using X-rays. In this research project, we propose to optimize this technique using FDA-approved X-ray imaging agents and demonstrate the efficacy in an animal heart attack model.

Most heart stem cell trials have injected stem cells into a vessel in the heart because this is a common method to diagnose and treat narrowed vessels in the heart. Yet, this method is very invasive and data suggests that stem cells may survive better if injected directly into the heart muscle by feeding a catheter from a blood vessel into the vessels in the heart. Using our new stem cell bubble technique, we propose to compare direct delivery to the heart muscle to a new delivery approach –direct injection from the chest wall into the sac that surrounds the heart. If successful, this new delivery method could provide a less invasive method to deliver stem cells that could be used repeatedly.

In addition, we will use a state-of-the-art imaging system that combines magnetic resonance imaging (MRI) with X-ray imaging. Thus, the specific aims are: 1. To develop a high-throughput, single-cell bubble technique that incorporates off-the-shelf X-ray contrast (Xµcaps); 2. To optimize MRI and X-ray imaging for direct heart muscle and sac delivery of Xµcaps; and 3. To test the efficacy of these Xµcaps in a swine heart attack model to provide data for FDA clinical trial approval. Optimization of the Xµcaps will be performed in Drs. Wang and Mao's labs with MRI and X-ray-visibility performed under the direction of Dr. Kraitchman. Biocompatibility of the Xµcaps will be performed in culture and live animals. Expertise in delivery device development and integration of X-ray-MRI platforms will be performed in concert with our Maryland industrial partner, Surgivision, Inc., a leader in the development of MR-compatible biopsy and delivery systems. The long-term goal of this project is to create a stem cell treatment for the heart with enhanced cell survival that can be delivered in a minimally invasive manner using clinical imaging systems.

Single Cell Microencapsulation For Ischemic Heart Disease Therapy

Principle Investigator: **Dara Kraitchman**
Institution: Johns Hopkins University
In collaboration with Surgivision, Inc.
Project Budget : $689,836

Methods to treat heart disease have improved over the last several decades and many more patients survive a heart attack. However, almost 13% of heart attack patients die within a year and almost 1/3 of heart attack patients experience another heart attack or heart failure. Many clinical trials have been performed to study the safety and effectiveness of stem cells as therapy to repair damaged hearts or lessen the likelihood that a person will develop a failing heart. But stem cell therapies are hampered by the fact that close to 95% of the stem cells die within 1 day to 1 week after they are delivered. As a result, it is not surprising that these trials have shown poor results in patients.

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Regulation of Neural and Neoplastic Stem Cells by Kruppel Like Transcription Factors

Principle Investigator: **John Laterra**
Institution: Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Project Budget : $689,836

The effective application of stem cell (SC)-based treatments for diseases of the central nervous system will require the ability to tightly control the growth and differentiation of therapeutic neural SCs (NSC). Advances are being made toward maintaining NSC multipotency (ability to differentiate into different neural cell types) in vitro and in defining culture conditions that induce differentiation into specific cell types of interest for therapy (e.g. neurons, oligodendrocytes). Obstacles such as scalability and controlling cell fate remain. Regulating NSC growth and differentiation after implantation to brain and spinal cord also remain major obstacles. Uncontrolled growth of poorly differentiated cells risks the formation of functionally irrelevant and even malignant tumors, a recognized risk of current induced pluripotent stem cell technology. Premature and misdirected differentiation risks repopulating the brain or spinal cord with insufficient numbers of irrelevant cell types. Neural differentiation strategies also hold great promise for treating brain cancers such as glioblastoma that contain stem-like cells (i.e. cancer SCs, CSCs) believed to play a dominant role in tumor growth, resistance to treatment, and recurrence.

Growing evidence shows that normal NSCs and neoplastic CSCs share signaling pathways that regulate their growth and cell-type specific differentiation. Therapeutic approaches based on modulating transcription factors (proteins that bind to DNA to directly regulate gene expression) show promise for their capacity to regulate SC differentiation for regenerative applications and to inhibit CSCs and their capacity to support tumor growth. Emerging evidence implicates a specific transcription factor family called Krüppel-like factors (KLFs) as regulators of neural cell generation and/or cancer. However, how KLFs actually work and their importance to neural SC biology remain relatively unknown. We recently found a link between two KLF family members (KLF9 and KLF4) and cell signaling mechanism that regulate both non-neoplastic NSCs and GBM-CSCs. We also found that KLF9 inhibits GBM-CSC growth, and induces GBM-CSC differentiation. This tumor suppressing activity of KLF9 was found to involve the direct inhibition of a gene called Notch1 that is known to regulate stem cells and certain cancers. This proposal is based on the hypothesis that KLF4 and KLF9 play important, distinct, and possibly opposing roles in regulating both NSCs and GBM-CSCs. The experiments will determine how KLF4 and KLF9 regulate NSCs and GBM-CSCs growth, differentiation, and context-dependent gene expression. Positive results from these experiments will provide vital information regarding how non-neoplastic and neoplastic neural stem cells are regulated. Findings will establish the basis for novel approaches directed at controlling stem cells for regenerative medicine and brain cancer therapy.

Remodeling the DNA Damage Response in Induced Pluripotent Stem Cells

Principle Investigator: **Feyruz Rassool**
Institution: University of Maryland, Baltimore
Project Budget : $662,998.30

Human embryonic stem cells (hESC) must maintain the integrity of the organism so that the genes encoding all the proteins for the propagation of life are faithfully transmitted from generation to generation. These processes are critical for the maintenance of genomic integrity of the organism. hESC have therefore evolved multiple mechanisms to protect their genome from various types of DNA damage: One important mechanism is to faithfully and correctly repair DNA that becomes damaged. If DNA is not repaired correctly, the cell can be induced to "commit suicide" and die, thus avoiding the danger of passing the damaged DNA to the next generation. The danger of allowing incorrectly repaired DNA damage to be carried to subsequent progeny is disease, premature aging and cancer.

Adult somatic cells can be induced to "dedifferentiate" into induced pluripotent stem cells (iPSC) and reprogram into cells of all three germ layers. IPSC have great potential in regenerative medicine and patient-specific iPSC have been derived not only for disease modeling but also as sources for cell replacement therapy. However, it is essential that data be accrued to prove that iPSC are functionally equivalent to human ESC (hESC) or are as safe as hESC. In this regard few studies have examined how well the DNA damage and repair response (DRR) is reprogrammed in iPSC. Importantly, our preliminary data shows that iPSC engineered using four essential factors, fully reprogram the DNA repair response, but demonstrate a partial apoptotic response compared to hESC. Thus, DDR is likely to provide critical markers in studies to determine the efficacy of reprogramming using non-viral methods and/or iPSC derived from different cell types.

In this study, assays for DNA damage and repair, well established in the Rassool laboratory, will be used to characterize the DNA damage/repair response in iPSC prepared using a wide repertoire of high quality non-viral and viral iPSC made from several tissue types in the Zambidis laboratory (at least >20 established lines), and compared with established hES cells and iPS parental controls. These cells will be studied for endogenous levels of DNA damage and repair and following treatment with agents that damage DNA. Importantly, we will characterize the molecular features of the processes used to induce cell death in hESC and iPSC. Regeneration or recovery of injured tissue of iPSC exposed to DNA damage IPSC exposed to a variety of DNA damaging agents will be used to determine whether this affects the efficiency of reprogramming cells into all into all three germ layers (endoderm, ectoderm, mesoderm) or the regeneration of tissue injury, compared with hESC. This understanding may allow us to prevent iPSC participating in regenerative processes from leading to disease, aging and cancer. The results of this study are critical for the safe use of iPSC as a source for cell replacement therapy in humans.

Intraarterial Targeted Delivery of Stem Cells to Brain Lesions Under MRI Monitoring

Principle Investigator: **Piotr Walczak**
Institution: Johns Hopkins University
In collaboration with Q Therapeutics
Project Budget : $689,999

Successful stem cell-based therapy of neurological disease depends on the efficient delivery of cells to brain area affected by disease. Direct injection of cells to brain tissue using small needle, which is the most commonly applied route of cell delivery, has serious disadvantages, namely, it is invasive and results in limited, non-uniform cell distribution. In a clinical cell therapy trial of Parkinson's disease patients, non-uniform engraftment led to serious side effects, forcing the investigators to halt the trial. Targeted, intraarterial cell delivery seems to be ideally suited to address this problem. However, techniques for efficient targeting and precise monitoring of cell delivery, to ensure the safety of this approach, are needed. Recognizing that intraarterial cell delivery has an advantage over the intravenous approach only if cells bind to the lesion endothelium during the first round of circulation in the bloodstream, before they would be filtered in lungs, we propose to engineer the cells to produce specific receptor on cell surface increasing their binding to brain endothelium activated by disease process. Realizing that intraarterial cell delivery with excessive engraftment could lead to blocking of cerebral blood flow and it can be considered for clinical application only if the safety of this approach is ensured, we propose to monitor the engraftment of stem cells, as well as cerebral blood flow and oxygenation, in real-time using magnetic resonance imaging. We will evaluate applicability of intraarterial cell delivery in two rat models of neurological disease: cerebral ischemia and model of multiple sclerosis. The experimental design will include lesion induction, cell engineering, intraarterial cell infusion, MRI, behavioral analysis and histology. If successful we will establish new method of stem cell delivery that is safe, efficient, and would likely find applications in many disciplines of medicine even outside of neurology.

Clinical-Grade CD34-iPSC for Hematologic And Cardiac Therapies

Principle Investigator: **Elias Zambidis**
Institution: Johns Hopkins University
In collaboration with Life Technologies, Inc.
Project Budget : $690,000

In this revised TEDCO proposal, we will work to derive and commercialize methods and reagents for reprogramming somatic cells into clinically safe patient-specific pluripotent stem cells for preclinical hematologic and cardiac studies. This will be a collaborative academic-biotechnology venture between a team of Investigators at the Johns Hopkins Institute for Cell Engineering, and the Pluripotent Stem Cell R&D Division of Life Technologies (http://www.lifetechnologies.com/about-life-technologies.html), a Frederick, Maryland-based biotechnology company (a recent merger between Invitrogen and Applied Biosystems) that specializes in developing current good manufacturing practice (cGMP)-grade reagents and media for stem cells.

The technology we will develop is an extremely efficient, nonviral, non-integrating approach discovered in our laboratory for generating high-quality human induced pluripotent stem cells (hiPSC) from human CD34+ blood-derived progenitors (CD34-iPSC). Our long-term goal is to develop methods for producing commercial grade CD34-iPSC that are completely devoid of malignant potential. Our Aims will demonstrate the potential of nonvirally reprogrammed CD34-iPSCs for generating two therapeutically important cell lineages: hematopoietic and cardiac. A secondary goal is to ultimately commercialize this novel CD34-iPSC technology in collaboration with our biotechnology partners.

EXPLORATORY RESEARCH ABSTRACTS

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MSCRF 2011 ANNUAL REPORT

Telomerase in Ex Vivo Expanded Hematopoietic Stem Cells

Principle Investigator: **Mary Armanios**
Institution: Johns Hopkins University
Project Budget : $230, 000

Telomeres are biological clocks in stem cells. Made up of repetitive DNA and proteins, telomeres protect the ends of chromosome. Because of the way DNA replicates, telomeres shorten as stem cells divide and when they reach a certain threshold, critically short telomeres cause stem cell loss. Telomerase is a remarkable enzyme that synthesizes new telomeres. Its levels are exquisitely regulated normally. We and others have shown that in humans that inherit one defective copy of telomerase, telomeres shorten and stem cell failure occurs in the bone marrow. The bone marrow failure is often recognized as part of a syndrome known as dyskeratosis congenita. This syndrome is a common cause of inherited bone marrow failure in children and adults. In these patients, bone marrow failure is progressive and often fatal because of the loss of blood producing stem cells. Bone marrow transplantation is the only curative treatment but is particularly toxic because these individuals have fragile reserves in organs outside the bone marrow. We have characterized a large registry of patients with dyskeratosis congenita who have telomerase deficiency. This Registry has provided significant insights into the role of telomerase in stem cell maintenance and disease. Our goal in this exploratory application is to identify novel, feasible approaches to rescue this defect. Recently there has been exciting progress made in identifying methods to expand the number of blood forming stem cells. Since patients with telomerase mutations have normal stem cells that are few in number, we propose to test this expansion method to rescue the stem cell failure due to telomerase deficiency. Our experiments hold promise for identifying a novel therapeutic strategy for an otherwise fatal syndrome. The experiments are also significant for defining the feasibility of other stem cell applications including bone marrow transplant.

Differentiation of Fibroblast-Derived Induced Pluripotent Cells Into Oligodendrocytes for Treatment of Neurological Disorders

Principle Investigator: **Visar Belegu**
Institution: Hugo W. Moser Research Institute at Kennedy Krieger, Inc.
Project Budget : $230,000

Spinal cord injury is a devastating condition, which imposes major individual and societal costs. The goal of our work is to promote functional recovery in patients following spinal cord injury by utilizing therapeutic approaches that promote remyelination. Remyelination from the transplanted cells is one mechanism that can induce partial functional recovery. In a rodent model of SCI, transplanted human embryonic stem cells (hESC) induce functional recovery, and a process know as remyelination is believed to play a role. However, significant ethical and safety concerns remain associated with the use of hESCs in a clinical setting; these, include the possibility of immune rejection, and the need for immunosuppression in a patient population that is at risk of life-threatening infections. The generation of induced pluripotent stem cells (iPSCs) is delivering on the promise of creating patient-specific cells for therapeutic transplantations. Unlike, hESCs, the use of iPSCs in transplantation-based therapeutic approaches does not face any immune related issues or ethical ones.

THE ROLE OF HMGA1 IN INTESTINAL STEM CELLS & THE GENERATION GUT-LIKE TISSUE

Principle Investigator: **Amy Belton**
Institution: Johns Hopkins University
Project Budget : $230, 000

Recent advances in stem cell research provide the extraordinary potential to model complex diseases and to develop strategies to repair or replace tissues damaged by disease. The overall goal of this proposal is to enhance our understanding of gut stem cells (both in normal gut and colon cancer) and to develop safe and effective stem cell technology to generate gut-like tissue. Colon cancer is a leading cause of morbidity and mortality worldwide and our studies will benefit this patient population. In fact, >1 million new cases are diagnosed each year and colon cancer is the 2nd cause of cancer mortality in the US and other developed countries. In addition, inflammatory bowel diseases are associated with colon cancer and loss of gut tissue; thus, these patients will also benefit from advances in regenerative medicine focused on the gut. The cellular pathways that are responsible for normal gut stem cells and the abnormal properties of colon cancer cells are poorly understood. Research is also needed to determine how colon cancer cells elude therapy and spread to distant sites in the body so that current therapies can be improved. Recent research indicates that these refractory cancer cells develop by co-opting cellular pathways active in normal stem cells, which thereby enables the cancer cells to resist treatment and spread beyond their primary sites. My preliminary studies performed during my MSCRF fellowship thus far indicate that the HMGA1 gene is important in the development of normal gut stem cells and colon cancer stem cells. Moreover, work from our group found that HMGA1 is highly expressed in aggressive colon cancer, human embryonic stem cells (hESCs), and cells engineered to behave like embryonic stem cells (also known as induced pluripotent stem cells or iPSCs). Like other stem cell factors, HMGA1 expression falls when stem cells differentiate. More recently, preliminary studies from our laboratory indicate that HMGA1 enhances the development of human iPSCs. Taken together, these findings strongly suggest that HMGA1 is critical in driving the stem cell phenotype, both in normal intestinal stem cells, colon cancer stem cells, hESCs, and iPSCs. Based on these results, we hypothesize that: 1.) HMGA1 is a key factor in intestinal stem cells and colon cancer stem cells, and, 2.) HMGA1 will enhance the derivation of gut-like tissue from hESCs and iPSCs. Using our unique reagents, we now propose to test these hypotheses with the following Specific Aims: 1.) Define the role of HMGA1 in normal and colon cancer stem cells, and, 2.) Develop safe stem cell technology to generate gut-like tissue. Our proposed studies will enhance our knowledge of intestinal stem cells and colon cancer stem cells. Results from our studies should also lead to the development of therapeutic strategies to target refractory colon cancer and regenerate gut-like tissue for diverse human diseases affecting this tissue.

A Preclinical Model of Acquired Aplastic Anemia Using Human Induced Pluripotent Stem Cells

Principle Investigator: **Robert Brodsky**
Institution: Johns Hopkins University
Project Budget : $230, 000

Acquired aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) and are closely related bone marrow failure disorders. In most cases AA is an autoimmune disease where the patient's own immune system attacks the bone marrow stem cells. Consequently, patients with AA have very low blood counts are prone to severe infections, bleeding and even death. Bone marrow transplantation (BMT) is the only potentially curative therapy but most patients don't have a suitable donor. PNH is caused by a mutation (PIGA gene) occurring in a blood stem cell. For reasons that are not completely understood, PNH occurs in patients with AA. Unfortunately, there are no suitable animal models to study why an autoimmune attack directed at a stem cell compartment harboring a minor population of PNH stem cells leads to growth and expansion of the PNH clone. The objective of this proposal is to develop the first ever model of AA using human induced pluripotent stem (iPS) cells. We plan to model AA by using T cells (the autoimmune cells that attack the patient's stem cells) from AA patients to target stem cells derived from differentiated iPS cells from the same patient. We will also introduce a PIGA mutation into the AA derived human iPS cells and, for the first time, be able to study directly whether PIGA mutant stem cells have a growth advantage in the setting of AA. Establishing an iPS model of AA should improve our understanding of the basic biology of AA and improve our understanding of why PNH stem cells seem to have a conditional growth advantage in the setting of AA. It may also serve as a model to develop novel drug therapies to treat AA. Our project is also highly relevant for the field of regenerative medicine. In future years, we hope to be able to grow enough stem cells outside of the body to be used for autologous bone marrow transplantation in order to treat patients with AA.

A Preclinical Model of Acquired Aplastic Anemia Using Human Induced Pluripotent Stem Cells

Principle Investigator: **Kan Cao**
Institution: University of Maryland, College Park
Project Budget : $230, 000

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by dramatic premature aging. Patients with HGPS appear normal at birth, but begin to display alopecia, growth retardation, bone abnormalities, osteoporosis, and sclerodermatous skin by age one. On average, death occurs at age 12 from heart attack or stroke. Classic HGPS is caused by a de novo point mutation in exon 11 (1824, C->T) of the LMNA gene, activating a cryptic splice donor and resulting in a mutant lamin A protein termed "progerin" that lacks the normal cleavage site to remove a C-terminal farnesyl group. The molecular mechanism underlying HGPS pathogenesis is mostly unknown. In addition, there is a remarkable degree of similarity between the conditions found in HGPS and in normal aging, including vascular disease, heart disease, stroke, and osteoporosis. We hypothesize that that abnormality and depletion of adult stem cells is a major factor in causing the tissue specific defects observed in HGPS. To test our hypothesis, we use a potent combination of the induced pluripotent stem cell (iPSC) technology with state-of-the-art cell biological, biochemical, and genomic approaches. Specifically, we plant to generate iPS cells from HGPS patient fibroblasts, and use them and their differentiated cells as models to study the molecular regulation of progerin/lamin A expression, and to test potential drug treatments. Our goal is three-fold: (1) to elucidate the molecular controls of lamin A and progerin expression in different tissues; (2) to develop and test new treatments for HGPS, and (3) to exploit our knowledge of HGPS to better understand cardiovascular disease and normal human aging.

Signaling Mechanisms Specifying Oligodendrocyte Development

Principle Investigator: Raghothama Chaerkady
Institution: Johns Hopkins University
Project Budget : $230, 000

The ability to derive cells of the oligodendroglial lineage from human embryonic stem cells (hESCs) with high efficiency opens novel prospects for cellular replacement strategies for demyelinating diseases such as multiple sclerosis and spinal cord injury. Indeed, several studies have already shown proof-of-principle in animal models for cell-based therapies. One of the important functions of oligodendrocytes (OLs) in the central nervous system is to insulate neurons by myelination. Although DNA microarray based experiments to identify such molecules have been carried out, determining the phosphorylation of proteins and their levels using a quantitative proteomics approach is the most direct way to identify molecular pathways that are specific for oligodendrocyte differentiation. Hence deep proteomic analysis is a promising strategy necessary to discover not only novel functional proteins but also establish the role of reversible phosphorylation events in signaling pathways

TiO2based enrichment of phosphopeptides and phosphotyrosine specific antibody affinity combined with high-resolution Fourier transform mass spectrometric methods will allow us to quantitate changes in very low abundant phosphopeptides specific to oligodendrocytes differentiation and functions to accomplish our goals.

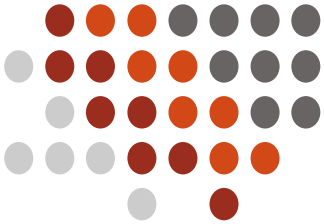
We hypothesize that several oligodendroglial system processes operating in a temporospatial fashion are established during differentiation of hESCs into OLs and based on our preliminary results, they can be detected utilizing our technology.

This study proposes validation and screening of specific phosphorylated proteins identified in exploratory studies using targeted multiple reaction monitoring (MRM) based assays. We will take the advantages of targeted mass spectrometry approach to validate candidate phosphoproteins identified in exploratory phase. This strategy is novel and powerful approach for simultaneous validation of many candidates in different stem cell lines. This also provides absolute quantitative information and lead to the development of universal assay methods that can be used by other researchers. The goal of this proposal is to utilize a quantitative phospho proteomic approach to study activated kinases and phosphoproteins in oligodendrocytes differentiated from human embryonic stem cells (ESCs). The proposal aims to characterize global changes in phosphorylation to identify new targets for clinical application. Identification of lineage specific phosphoproteins could be used to develop new drugs that could treat spinal cord injuries or demyelinating diseases, such as multiple sclerosis.

MicroRNA Regulators of Ex Vivo Human Hematopoietic Stem

Principle Investigator: Xiaochun Chen
Institution: University of Maryland, Baltimore
Project Budget : $230, 000

A limitation of hematopoietic (blood-forming) stem cell (HSC) transplantation is insufficient numbers of stem cells from the donor, especially when the donor tissue is umbilical cord blood. Developing a method to expand the numbers of donor HSCs has been a long sought goal in hematopoietic research. Many attempts have been made using cytokine cocktails, growth factors, and stromal cell support, but HSC expansion remains elusive. MicroRNAs (miRs) are a new class of intracellular regulators that play important roles in many cellular processes, such as cell proliferation and differentiation. Recently miR-125a and miR-125b were found to expand HSCs by blocking programmed cell death. In this project, I propose to identify existing drugs (now used for other purposes) which can increase the levels of those two candidate miRs, as a novel HSC expansion strategy. Mining new uses for old drugs has been an increasingly important approach in drug development. Because the safety data on such 're-positioned' drugs are already available, the time and cost to move these laboratory discoveries to patients is greatly reduced. During his postdoctoral research training, the Principal Investigator of this project has succeeded in rapid screening to reveal such re-positioned drugs. MSCRF's funding of this proposal would support (a) laboratory research to discover drugs that raise the levels of ('upregulate') those two miRs and (b) initial preclinical studies of those new agents for their ability to increase HSC numbers. MSCRF funding would move us toward Phase I/II clinical trials of these re-positioned drugs.



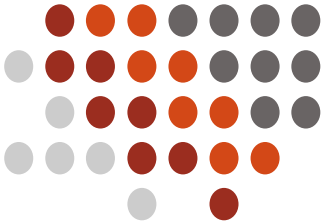
Stromal Hedgehog Signaling In B-Lymphoid Differentiation From Human Adult Stem Cells

Principle Investigator: Stephen Desiderio
Institution: Johns Hopkins University
Project Budget : $200, 000

Antibodies are natural substances that recognize and intercept bacteria, viruses and other foreign substances that threaten health. They are the most effective line of defense against infectious agents like influenza and the toxic products of bacteria like diphtheria. Antibodies often work by interrupting the multiplication of viruses or by blocking toxic bacterial products so that they cannot cause damage. Vaccines are an example of a procedure used to trigger production of antibodies that are able to recognize and protect against a particular foreign target. More recently the ability to engineer antibodies through recombinant DNA technologies have given rise to a new class of treatments for cancers, inflammatory and autoimmune diseases like rheumatoid arthritis, asthma and heart disease.

Antibodies are normally made by a specialized type of white blood cell called a B lymphocyte or B cell. The ability to efficiently produce B cells from human stem cells would make it feasible to develop novel, cell-based treatments based on the manipulation of B cells outside of the body.

For example, stem cells could be steered to develop into B cells capable of producing antibodies engineered to help eliminate a particular virus or cancer cell. The generation of B lymphocytes from human adult stem cells, however, is not efficient. For B cells to develop they require close contact with a supporting cell called a stromal cell. Our laboratory has recently found that the activity of a specific cell signal in stromal cells (called the hedgehog (Hh) signal) is required for mouse stem cells to efficiently give rise to B cells. Building on these findings, we now propose to test whether Hh signals in stromal cells also promote the generation of B cells from human adult stem cells or from human adult cells that have been reprogrammed to become stem cells. The studies we propose may provide a new way to stimulate the production of human B lymphocytes from stem cells. If successful, this project is expected to accelerate the development of a new generation of cell-based treatments in which patients are supplied B lymphocytes, derived from their own tissues, that have been programmed to produce antibodies tailored to their needs.



Reprogramming of Satellite Cells and Human Mesenchymal Stem Cells for Muscle Repair

Principle Investigator: Shao Jun Du
Institution: University of Maryland, Baltimore
Project Budget : $200, 000

The goal of this study is to generate muscle stem cells that can be used to treat skeletal muscle diseases such as Duchenne muscular dystrophy (DMD). Skeletal muscle damaged by injury or by degenerative diseases such as DMD is able to regenerate new muscle fibers. Muscle regeneration relies on muscle stem cells, namely satellite cells. Although muscle repair by satellite cells has been observed in the early phase of DMD, the satellite cell pool is quickly depleted during childhood of DMD patients, which leads to progressive muscle degeneration in DMD patients during late adolescence. Despite intensive efforts to develop treatment for DMD, currently there is no cure for this devastating disease. Cell-based tissue regeneration using muscle stem cells offers a new hope in treatment of DMD.

Satellite cells represent the natural choice in cellular therapeutics for DMD because of their intrinsic ability to form new muscles. The muscle microenvironment enables freshly isolated satellite cells to contribute extensively to skeletal muscle regeneration and can also re-populate the satellite cell pool when transplanted into muscles. This property is retained if stem cells are immediately transplanted from one individual to another following isolation, but it is lost quickly as the stem cells are plated in culture for expansion. The dramatic reduction of regenerative capacity could be caused by the rapid differentiation of satellite cells in culture.

Recent studies showed that microRNAs (a short single-strand RNA) play important roles in satellite cell self-renewal and differentiation. microRNAs control satellite cell renewal and differentiation via the downregulation of Pax3 and Pax7, two master regulators that define the muscle stem cells. We hypothesize that by modulating the function of microRNAs, we will be able to increase the Pax3 and Pax7 expression and consequently muscle stem cell regeneration. Our first objective of this study is to identify and characterize microRNAs involved in regulation of Pax3 and Pax7 expression, and to enhance the regenerative capacity of cultured satellite cells for regenerative medicine.

Although satellite cells are the natural choice for cell-based therapy, the limited supply and poor regeneration capacity in culture has limited their application in regenerative medicine. Human mesenchymal stem cells (MSCs), on the other hand, are remarkable in that they possess a large capacity for self-renewal, while maintaining their multipotency. Human mesenchymal stem cells have the potential to differentiate into cardiac and skeletal muscle cells. However, the application of mesenchymal stem cells in muscle regeneration has been hampered by their poor differentiation into the muscle lineage. We hypothesize that ectopic expression of Pax7 could convert mesenchymal stem cells into a muscle cell lineage. Our second objective is to reprogram mesenchymal stem cells into muscle stem cells via genetic reprogramming.

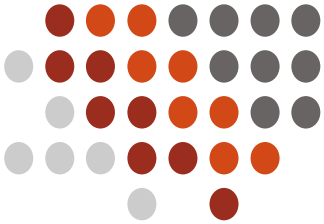
Alteration In Neural Fate In Schizophrenia

Principle Investigator: Koko Ishizuka
Institution: Johns Hopkins University
Project Budget : $230, 000

Schizophrenia (SZ) is a severe mental illness that results in abnormal mental functions and significant social disability in affected individuals. Although many lines of evidence have indicated that disturbances during early brain development may induce SZ, mechanistic understanding of the disease is not well developed. One major limitation that has blocked the progress is the difficulty of accessing relevant tissues/cells for study. First, because SZ is a brain disease, it is almost impossible to obtain biopsies from brains or neurons of central nervous system origin. Second, because the onset is in relatively young subjects (young adulthood), there is no guarantee that autopsied brains from aged patients with long-term medication reflect disease mechanisms. Therefore, our program has systematically collected accessible peripheral tissues and cells from patients with SZ as well as normal controls, and established neuronal cell lines from them with cell engineering technology.

Meanwhile, we found that one specific chemical modification on DISC1 protein, a major susceptibility factor for SZ regulates the function of DISC1 during brain development.

In this study, we will investigate the trait of SZ, such as cilia, cell organelles important for brain development and the specific chemical modification on DISC1 protein in the neuronal cell lines from patients with SZ. This study can lead to translational potential. Once we find the biological differences in neuronal cells between SZ and controls, this characteristic can be used for a biomarker for SZ, which can be utilized for diagnosis, especially useful in early diagnosis. To detect patients at the initial disease stage provides an opportunity for early, potentially preventative, interventions. Furthermore, better understanding of biological mechanisms of the disease can facilitate novel etiology-based drug discovery.



Human Stem Repair of Epithelial Tissue

Principle Investigator: Saul Sharkis
Institution: Johns Hopkins University
In collaboration with Quality Biological, Inc.
Project Budget : $230, 000

The use of adult stem cells for regeneration/repair of injured tissue has been demonstrated using both animal and human stem cells. In the hematopoietic system bone marrow transplants have been used for many years for treatment of hematological malignancies, bone marrow failure conditions and other hematological and autoimmune disorders. We have demonstrated in mice that stem cells isolated from the bone marrow by functional selection appear more primitive than stem cells selected by phenotypic markers. In order to utilize the most efficacious stem cell for repair of injured or diseased tissue it is necessary to select the most primitive and most multipotent stem cell for the repair. We plan to isolate human bone marrow derived stem cells (BMSC) to establish the purity and potency of these cells so they may be used for regenerative medical therapy. We will perform limiting dilution analysis of the selected cells and if necessary competitive repopulation against phenotypically isolated stem cells to establish the engraftment potential (purity) and self renewal potential (potency) of the functionally isolated cells. We will use properties such as quiescence; homing and/or aldehyde dehydrogenase content to positively select this population from lineage depleted anonymous human bone marrow samples. Thus our first specific aim is to isolate primitive human BMSC for the purpose of using these cells in clinical cellular therapy. Once we have established that our methods of selection provide an early BMSC we will attempt to treat a model of diabetes in immunodeficient mice with these cells to repair injury to epithelial tissue (i.e. beta cells) of the pancreas . Diabetes is a global problem with both severe physical and economic damage. In the United States alone over 20 million people are affected by this disease. In Maryland 5-7 % of the public is affected by this disease. The disease results from loss of functioning beta cells of the pancreas. We have data the indicates in the mouse that rare multipotent stem cells can reverse the metabolic damage associated with diabetes and in this proposal plan to demonstrate that transplantation of purified human BMSC can regenerate beta cells and correct the metabolic glucose dysfunction. Thus we will treat immunodeficient NSG mice with streptozotocin which chemically destroys beta cells of the pancreas. Three days later the mice will receive either phenotypically of functionally selected BMSC. Animals will be monitored for up to 6 months for blood glucose levels. At 6 months the mice will be sacrificed and the pancreatic and liver tissue examined for evidence of human epithelial cells which produce insulin. If engraftment and conversion of BMSC into epithelial tissue occurs as we have demonstrated in the mouse we can conclude that the BMSC can be used for transplant therapy in diabetes.

Stem Cell Therapy for Traumatic Brain Injury

Principle Investigator: Paul Yarowsky
Institution: University of Maryland, Baltimore
Project Budget : $230, 000

Traumatic brain injury (TBI) affects 1.5 million Americans a year; including over 3,000 in Maryland. When the brain is injured, nerve cells near the site of injury die due to the initial trauma and interruption of blood flow. Secondary damage occurs as neighboring tissue is injured, leading to a larger area of damage. This damage happens to both neurons, the electrically active cells, and oligodendrocytes, the cells which makes the myelin insulation around neurons. A TBI patient typically loses cognitive function associated with the damage. Currently, no treatments have been shown to be beneficial in alleviating the problems following even a mild TBI. Neural stem cells (NSCs) provide a cell population that is promising as a therapeutic for neurotrauma. One idea is that transplanting NSCs into an injury would provide "cell replacement"; the stem cells would differentiate into new neurons and new oligodendrocytes and fill in for lost host cells. Human NSCs have been used in rodent models of TBI, showing that NSCs integrate with the host, and can restore some lost function. However, fundamental issues such as the delivery of stem cells to regions of injury within the brain as well as their survival remain to be resolved. The feasibility of this strategy depends upon the extent to which transplanted neural stem cells will survive, differentiate, and migrate to their appropriate positions and act as biological pumps for delivering vital growth factors or proteins that are lost in these disorders. But a daunting problem exists in devising such a therapy, viz., how to deliver stem cells.

Our solution is by using directed magnetic fields and â€œmagnetizingâ€ the stem cells. We propose to develop a method of delivering iron oxide (ferumoxide, Feridex) labeled stem cells to specific areas of the cortex or to the entire spinal cord injured in TBI. In a rodent model of TBI, we will determine, if Feridex-labeled stem cells magnetized and containing a gene that allows the cells to migrate, can be localized at the site of lesion in the ipsilateral cortex by injection into the ipsilateral lateral ventricle. We will use production of these proteins as markers for the location and function of the stem cells in the magnetic field to verify our targeting approach. After injection of the stem cells, magnets will be placed either directly over the same side of the cortex (the stem cells are injected into the lateral ventricle; Specific Aim A). In Specific Aim B, we will determine repeat our studies but determine whether transplanted Feridex-labeled stem cells NPCs can be localized in the cerebral cortex at the site of lesion with intra-arterial injection, a less invasive procedure than intraventricular injection. We hypothesize that this method of magnetic targeting of stem cells will allow for accurate spatial delivery of stem cells.

Clinical Drug Screening Using Patient Specific Human iPSCs

Principle Investigator: Zhaohui Ye
Institution: Johns Hopkins University
Project Budget : $230, 000

Human induced pluripotent stem (iPS) cells provide unprecedented opportunities for regenerative medicine applications. In addition to cell replacement therapy and disease modeling, drug discovery is another major application through which the iPS technology has the potential to accelerate the delivery of new therapies. One of the major limitations of the conventional drug screening and testing is the utilization of tissue-cultured animal or human cell lines which do not represent the cells in human body. This adds significant burden to the subsequent lengthy and much more costly animal testing and clinical trials. Disease-specific iPS cells may represent a novel and more relevant source. In this proposal we will explore the utility of iPS cells in drug screening and in pre-clinical drug testing using disease-specific iPS cells generated from polycythemia vera (PV) patients.

The major clinical feature of patients suffering from PV is the over-production of red blood cells and increased blood viscosity which can be life-threatening. Effective drug treatment for PV is currently lacking while phlebotomy has been used to decrease blood thickness. PV is caused by rare blood stem cells with mutations. The lack of methods to obtain and to expand these cells in the laboratories made it difficult to study the disease mechanism or to develop effective treatment. To provide a solution to this problem, we have generated PV patient-specific iPS cells that can be maintained and expanded in the laboratory. More importantly, when compared to iPS cells generated from healthy donors, these cells have the capacity of over-producing red blood cells in a dish, just like the stem cells in PV patients. This provides a highly disease-relevant system for developing drug treatments that have been limited by the shortage of expandable diseased stem cells from the patients.

In this proposal, we will use these disease-specific cells to test multiple pharmaceutical compounds currently undergoing clinical trials in order to determine the feasibility of these iPS cells in pre-clinical drug testing. We will also screen a Clinical Compound Library with these renewable cells to discover new drugs that may effectively block the over production of red blood cells. Success of the project will not only provide new treatment for patients suffering from PV, more importantly it will establish iPS technologies as novel and effective platforms for drug screening and development. Ultimately, we anticipate it would lead to significantly reduced costs in drug development and more effective treatment for various diseases.

POST DOCTORAL FELLOWSHIP RESEARCH ABSTRACTS

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MSCRF 2011 ANNUAL REPORT

Encapsulated Human Mesenchymal Stem Cells for Improved Islet Cell Therapy

Principle Investigator: Dian Afrin
Institution: Johns Hopkins University
In collaboration with Pearl Lifescience Partners, LLC
Project Budget : $110, 000

We propose a new strategy to improve pancreatic beta islet therapy using human mesenchymal stem cells (HMSCs) and a microencapsulation method. This approach holds good potential for the promotion of islet angiogenesis, the regulation of immune responses without the use of immunosuppressive drugs, and the enhancement of islet graft therapeutic function. Pancreatic cells were first encapsulated in alginate beads gelled by 20 mM Ba2+ and then cross-linked by addition of a 0.05% protamine sulfate solution (clinical grade) and further cross-linked by addition of a second layer of alginate. Considering that the difference in the in vitro and in vivo effects of HMSCs, we proceeded to the animal study. Islet grafts typically suffer from hypoxia before angiogenesis takes place. In order to solve this problem, we investigated the effect of anti-hypoxic gene HIF1a by transducing HMSCs with a HIF1a gene (HMSCs+HIF1a). We have successfully established a protocol to transduce HMSCs with an adenovirus carrying HIF1a gene with a transduction rate close to 100%. We also studied the effect of co-encapsulating Perfluorooctyl Bromide (PFOB) emulsions. The use of PFOB has been extensively explored as an artificial blood and we predict that PFOB can serve as an oxygen sink to the encapsulated cells. Immunocompetent diabetic C57BL/6 mice were used as our model. Diabetes was induced by serial injections of streptozotocin, a toxin that destroys insulin-producing beta cells. Human islets were isolated from cadaveric donors. We subcutaneously transplanted 3000 microencapsulated human islets carrying luciferase gene with or without HMSCs (150 per islet) to the abdomen of diabetic mice. The viability of islet graft was monitored by bioluminescence imaging twice every week. Diabetic mice (n=3) received the following microencapsulated grafts: group 1: islets+HMSCs+HIF1a+PFOB; group 2: islets+HMSCs+HIF1a; group 3: islets+HMSCs+PFOB; group 4: islets+HMSCs; and group 5 (control): islets. Up to 30 days post-engraftment, 3 out of 3 mice in group 1 showed BLI signals, 2 out 3 mice in group 2, 3 out of 3 mice in group 3, 1 out of 3 mice in group 4, and 1 out of 3 mice in group 5. We plan to keep monitoring the BLI signals of the grafts until graft death (no signal) is detected. At this point, we will sacrifice the mice and extract the grafts. Immunohistology will be performed to study angiogenesis and immune rejections in the grafts.

Characterizing Notch Signaling in Bladder Cancer Stem Cells

Principle Investigator: William Brandt
Institution: Johns Hopkins University
Project Budget : $110, 000

Urothelial carcinoma (UroCa) is the 5 most prevalent type of cancer in industrialized countries, with more than 300,000 diagnoses and more than 100,000 deaths annually. This mortality stems from chemoresistant metastatic disease, which develops in almost all cases despite a high rate of initial chemotherapeutic response. We have preliminary data that Notch signaling regulates UroCa stem cells (UroCSCs), and plan to investigate this through over expression and suppression of Notch pathway members specifically and separately in human UroCSCs, UroCICs (intermediate cells), and the stroma in an attempt to deplete this treatment-resistant population of cells.

Microarray analysis followed by Analysis of Functional Annotation found enrichment of genes associated with angiogenesis, apoptosis, migration, motility, and proliferation in genes differentially expressed in the basal-like UroCSC population. These genes were also associated with enriched developmental signaling pathways including Jak-STAT, Wnt, EGFR/ErbB, and most notably, Notch.

For Aim 1, I hypothesize that Notch signaling drives differentiation, and that Notch activation in human UroCSCs will therefore inhibit the ability to initiate and maintain tumor growth by depleting the chemo-resistance UroCSC population. Notch inactivation should prevent UroCSC differentiation reducing overall tumor growth. In Aim 2, I hypothesize that Notch over-expression in mouse embryonic fibroblasts (MEFs) will result in increased stromal plasticity, an enhanced ability to support tumor growth, and increased angiogenesis. MEF's lacking RBP-J will delay or inhibit tumor growth, with less arteriogenesis.

Detailed monitoring and analysis of each cellular compartment over time should elucidate not only the role of UroCSCs in bladder cancer, but also the requirement of cancer stem cells to interact with stroma to drive tumor growth. Understanding the importance of these interactions, both from the perspective that UroCSCs are the driver (Aim 1) or that the stroma drives growth (Aim 2), will be critical for targeted therapy moving forward.

Understanding The Role Of CYFIP1 Deletion/Duplication In Schizophrenia And Autism

Principle Investigator: Namshik Kim
Institution: Johns Hopkins University
Project Budget : $110, 000

Autism is a neuro-psychiatric disorder characterized by markedly abnormal social interaction and communication skills with an early onset. Schizophrenia is also a neuro-psychiatric disorder characterized by mental impairments in the perception expression of reality and by social dysfunction. While the exact etiology is not known, both disorders have been considered as diseases with a neural developmental origin. In human genetic association studies, a deletion at chromosome 15q11.2, which includes Cyfip1 gene, is significantly associated with schizophrenia. Interestingly, duplications of the Cyfip1 containing region from 11q11.2 to 15q13 are well-documented risk factors for autism. These data suggest that Cyfip1 may play dual roles as a causative factor for schizophrenia and autism in a dose -dependent manner. Cyfip1 was originally identified as a fragile X mental retardation protein (FMRP1) binding protein. Previous studies have shown that Cyfip1 controls synaptic remodeling by regulating Rac1-mediated cytoskeleton reorganization and local protein translation via FMRP1. The specific functional contributions of Cyfip1 to these psychiatric diseases are largely unknown. Mimicking such a chromosomal deletion and duplication of human patients is very difficult and time consuming with conventional ES cell based gene targeting technology in mice. Therefore, alternative approach using patient derived iPSC could be a major breakthrough to tackle this issue. The goal of this study is to understand the role of Cyfip1 deletion/duplication in the development of schizophrenia/autism, respectively, and their underlying mechanisms with 2 specific aims.

Aim 1: Derivation of iPSCs from patients with Cyfip1 deletion/duplication and in vitro characterization.

Aim 2: In vivo characterization of neurons derived from iPSCs with Cyfip1 deletion/duplication after transplantation.

Our study of the cellular and developmental roles of Cyfip1 deletion and duplication may provide molecular insights into the functional roles of Cyfip1 as a putative etiological factor in both schizophrenia and autism, and may provide the basis for the identification of new targets for drug development.

A High Content Screen for Small Molecules that Promote Stem Cell differentiation into Retinal Pigmented Epithelial Cells for Potential Treatment of Age-Related Macular Degeneration and Other Retinal Degenerations

Principle Investigator: Julien Maruotti
Institution: Johns Hopkins University
Project Budget : $110, 000

Nearly 1.75 million U.S. citizens are affected by Age-related macular degeneration (AMD) now and with America's aging population this number is expected to reach almost 3 million by 2020. AMD is a devastating disease of the retina that eventually leads to definitive blindness: it affects the retinal pigmented-epithelial cells (RPE) — a layer of cells surrounding the retina. When RPE cells do not function properly, photoreceptor cells, the cells in the eye that capture light, begin to die and vision is lost.

Cell-based transplantation strategies offer the promise of being able to restore RPE cells thus potentially limiting vision loss. Therefore, establishing an abundant and quality source of donor cells is of utmost importance. Human induced pluripotent stem cells (hiPSCs) may prove suitable for this purpose: significant advances have recently been made in inducing the differentiation of hiPSCs toward an RPE-like cell fate. Nevertheless, the length and efficiency of these methods are still not optimal. We propose here to use High Throughput Flow Cytometry to identify small molecules or factors that could improve this process.

Monolayer differentiation of hiPS cells into RPE cells will be performed based on published protocols. We will then screen for compounds or factors that can specifically promote induction of RPE progenitor cells. Using a robotic, flow cytometry based system we will be looking for molecules that can enhance the frequency and /or degree of differentiation into RPE-like cells by following expression of early RPE-specific cell markers.

Telomere Recombination Mechanisms In Mesenchymal Stem Cells

Principle Investigator: Tammy Morrish
Institution: Johns Hopkins University
Project Budget : $110, 000

Telomeres are typically maintained by telomerase, however recombination-based mechanisms also likely contribute to telomere maintenance. Telomere length maintenance prevents loss of genetic information and is critical for cell viability. In tumors, continued cell growth requires telomere maintenance yet some tumors can grow without telomerase. Mutations in telomerase lead to human bone marrow failure diseases, including aplastic anemia and dyskeratosis congenita. Tumors that grow in the absence of telomerase typically are derived from cells of mesenchymal cell origin, including sarcomas and certain types of brain tumors. It is not certain why telomere recombination mechanisms, instead of telomerase are preferentially utilized in tumors of these origins. From our previous studies we observed that in primary cells with short telomeres, mTR-/- primary bone marrow cells had a greater amount of telomere recombination compared to mTR-/- splenocytes with short telomeres. Our goal is to understand why certain types of tumors more readily utilize recombination based mechanisms of telomere maintenance. We hypothesize that telomere recombination mechanisms may normally contribute to telomere maintenance in some primary cell types such as mesenchymal stem cells compared to cells of hematopoietic origin. In this proposal we will study primary bone marrow from both human and mice to determine whether telomere recombination is more frequent in mesenchymal stem cells (MSCs). In addition we will use shRNAs to knockdown certain recombination genes to examine whether recombination normally contributes to telomere maintenance in MSCs compared to hematopoietic stem cells (HSCs). Finally we will examine whether tumors derived from MSCs require recombination genes for tumor initiation and growth. These studies will determine whether recombination mechanisms can contribute to telomere maintenance in certain types of adult stem cells. Furthermore, disruption of these recombination pathways in tumor cells may provide treatment for certain types of tumors.

Modulating HGF/c-Met Signaling in Human Normal and Neo Plastic Stem Cells

Principle Investigator: Prakash Rath
Institution: Johns Hopkins University
Project Budget : $110, 000

Glioblastoma multiforme (GBM) is a fatal brain tumor where the average survival is approximately 14 months post-diagnosis. GBMs are composed of heterogeneous cell populations and there is an increasing interest in targeting the stem-like tumor-initiating pool of cells (TICs) within GBMs, as this subset of cells has been shown to be resistant to conventional therapies and associated with tumor recurrence. This resistance suggests that developing therapies directed against the tumor stem-like cell population may be a promising approach for effectively treating GBMs, particularly for preventing or delaying tumor recurrence. Hepatocyte Growth Factor (HGF) and its tyrosine kinase receptor c-Met is a promising molecular target for investigations in GBMs. HGF/c-Met expression levels correlate with poor prognoses and Met signaling is well documented to be associated with tumor proliferation, angiogenesis, and invasion.

Previously we have shown that anti-HGF therapy using the monoclonal antibody L2G7 inhibits the progression of orthotopic xenografts derived from HGF+/c-Met+ GBM cell lines via inducing apoptosis, and inhibiting tumor cell proliferation and angiogenesis. Here we tested the hypothesis that Met signaling pathway inhibitors deplete the stem-like cell phenotype and the tumor initiating capability in GBM cell lines and primary GBM xenografts.

To examine this, we developed a xenograft model for investigating the stem cell phenotype from GBM cell lines, where U87 xenografts were able to confer a stem-like phenotype. Additionally, we demonstrated through this model system that L2G7 treatment of subcutaneous xenografts arrested U87 tumor growth ($N=5$ $p<0.0001$), which displayed a rebound growth phenomenon upon treatment withdrawal. We tested additional primary GBM xenografts lines, which similarly showed significant decrease ($p<0.05$) in subcutaneous tumor volume upon treatment with the mAb L2G7 or the anti-met inhibitor PF2341006. Neurosphere growth assay and protein expression corroborated with the decrease in stem cell markers in treated tumors. Tumor initiation experiments with U87 cells showed a marked depletion in the treatment group, indicating a direct effect on the stem cell-like population.

These findings show that the approach for treating GBMs with anti-met signaling agents, that focuses on targeting the stem cell-like populations, is a promising therapy needs to be further investigated and may result in a better clinical outcome.

HMGA Proteins in Stem Cell Survival and Induced Pluripotency

Principle Investigator: Sandeep Shah
Institution: Johns Hopkins University
In collaboration with Life Technologies, Inc.
Project Budget : $110, 000

This proposal is directed at elucidating molecular networks important in maintaining a stem cell phenotype to facilitate the production of patient-derived, stem-like cells for regenerative medicine and disease modeling. Previous studies have identified a handful of pluripotency genes in human embryonic stem (hESCs), which led to the ability to reprogram normal adult cells into cells that resemble hESCs, called induced pluripotent stem cells (iPSCs). Unfortunately, the efficiency of induced pluripotency is exceedingly low because most cells fail to become fully reprogrammed. Moreover, "partially" or aberrantly reprogrammed cells can exhibit characteristics of cancer cells with invasive properties when implanted in immunosuppressed mice1. Thus, a better understanding of mechanisms that drive a stem cell state is urgently needed so that iPSCs can be safely used in the clinic. Recent studies from our group suggest that subtle differences in gene and protein expression patterns account for the different phenotypes observed in cancer, hESCs, and iPSCs. Here, our focus is the HMGA1 and HMGA2 genes, which are highly expressed during embryogenesis and enriched in hESCs, iPSCs, and high-grade cancer cells2. These genes encode chromatin remodeling proteins that modulate gene expression2. Like the stem cell factors NANOG and SOX2, HMGA1 and HMGA2 expression falls when hESCs differentiate (see below). My recent preliminary studies indicate that both HMGA1 and HMGA2 significantly enhance the derivation of iPSCs (see below). In addition, we found that: 1) Forced expression of HMGA1 blocks neural differentiation in hESCs, 2) HMGA1 induces expression of LIN28 and other pluripotency genes, while it represses a let-7 differentiation pathway, 3) HMGA1 is expressed at similar levels in hESCs and fully reprogrammed iPSCs, with lower levels in partially reprogrammed iPSC clones and cancer cells. Based on these exciting findings, we hypothesize that: 1) HMGA proteins drive stem cell properties in hESCs and promote cellular reprogramming in iPSCs, and, 2) HMGA proteins regulate expression of specific genes to maintain an undifferentiated, stem cell phenotype. Using our unique resources, we now propose to test our hypotheses and to identify critical networks induced by HMGA proteins that enforce the stem cell phenotype in normal hESCs and fully reprogrammed iPSC clones with the following specific aims: Aim 1: Define the role of HMGA proteins in the derivation of iPSCs and, Aim 2: Determine the mechanisms by which HMGA1 promotes iPSC generation.

Optimization of the 3D Scaffolds with Enzyme and Peptide for Osteogenic Differentiation in Stem Cells

Principle Investigator: Shobana Shanmugasundaram
Institution: University of Maryland, Baltimore
In collaboration with Biosurface Engineering Techologies, Inc.
Project Budget : $110, 000

Our long-term programmatic goal is to develop a stem cell-based tissue engineered "bone-on-demand" scaffold for bone regeneration. Current therapeutic treatments for bone repair are not yet fully satisfactory either due to the unsuccessful recruitment of cells at the injury site or mechanical integrity of the scaffold. Recent studies have shown that human bone marrow-derived mesenchymal stem cells (hBMSC) can potentially be mobilized into the circulation in response to injury signals and exert their reparative effects at the site of injury. It has been postulated that a "smart" scaffold that can trigger hBMSC homing at the injury site as well as play a role in the natural healing process to attract hBMSC would revolutionize the field of stem cell bioengineering. Elaborating on our previous results and published data, we hypothesize that the osteogenic differentiation in hBMSC can be potentiated by nanofibrous composite scaffolds of collagen type I and hydroxyapatite/b-tricalcium phosphate coupled with the osteoinductive constituents such as fibronectin, tissue transglutaminase and the BMP peptide analog, B2A. The following Specific Aims are proposed to achieve the goal of augmenting hBMSC osteogenesis for bone regeneration:

To develop a novel osteoconductive, bone matrix mimicking collagen I – ceramic scaffold for hBMSC osteogenesis: We hypothesize that hBMSC osteogenesis will be enhanced on a composite scaffold that mimics the structural properties of the bone ECM. To test this hypothesis, we will incorporate HA/bTCP at varied concentration, characterize thermal and physical properties of the scaffold, and analyze their functional activity on supporting hBMSC osteogenesis using the methods previously established by our group.

To enhance the osteogenic differentiation of hBMSC on collagen I- HA/bTCP composite scaffold by fibronectin (FN), transglutaminase2 (TG2) and B2A:

We hypothesized that incorporation of chemoattractive signals like TG2 and B2A would complement the effects of scaffold resulting from Aim 1 in augmenting hBMSC osteogenesis. The hypothesis will be evaluated by employing an iterative incorporation of fibronectin, TG2 and B2A on the scaffolds followed by quantification of the incorporation of FN and TG2 by ELISA and the release profile of B2A by BMP-dependent transcription using stable Cos-7 cell line expressing BMP-sensitive luciferase reporter.

To identify the molecular mechanism of osteogenic differentiation in hBMSC on composite scaffolds: Based on our previous findings, we hypothesize that TG2 and FAK dependent pathways will be relevant for hBMSC osteogenesis on composite scaffolds. To test the hypothesis, we will use pharmacological agents to mimic the effect of TG2 and FAK on hBMSC osteogenesis.

The proposed experiments will evaluate a novel approach of augmenting osteogenesis in human BMSC through design of a biomimetic scaffold that recreates the extracellular microenvironment of bone and is enhanced by TG2 and B2A incorporation. We expect that the presence of TG2 in the engineered scaffold would promote osteoblast differentiation in hBMSC via (1) cross-linking collagen type I enhancing its mechanical properties, (2) work in synergy with fibronectin and B2A enhancing cell attachment, proliferation, differentiation of hBMSC as well as amplify bone formation. The pharmacological approach will complement the TG2 mediated hBMSC osteogenesis on composite scaffolds which would add efficacy in a clinical setting.

Understanding the Mechanism of a Schizophrenia Susceptibility Gene DISC1 in Regulating Synapse Development Using Patient-Derived iPSCs

Principle Investigator: Juan Song
Institution: University of Maryland, Baltimore
In collaboration with Life Technologies, Inc.
Project Budget : $110, 000

Schizophrenia, a mental illness affecting more than 2.7 million people in the United States, has devastating effects on human thoughts, emotion and expression. DISC1 (Disrupted in Schizophrenia-1), one of the most prominent schizophrenia susceptibility genes, has been shown to affect multiple aspects of neuronal development during embryonic, early postnatal and adult neurogenesis in studies using animal models. However, how DISC1 dysfunction results in the clinical manifestation of a large set of severe psychological symptoms is largely unknown. The goal of this study is to understand the function of DISC1, in regulating human neural development, focusing on synapse formation and integration. The proposed study aims to test a novel hypothesis that DISC1 interacts with depolarizing GABA signaling in regulating synapse development and synaptic integration of iPSC-derived neurons. The hypothesis is built upon a series of findings we have made in the past on the characterization of the developmental processes of new neurons in the adult mouse hippocampus and its regulation by DISC1 and GABA signaling. In this proposal, I will take advantage of human induced pluripotent stem cells (iPSCs) as a model to investigate the interaction of DISC1 and GABA signaling in human neural development. Our lab has already generated iPSCs from schizophrenia patients with DISC1 mutation and control subjects. I will first differentiate these iPSCs into neuronal progenitor cells and transplant them into mouse embryos via in utero transplantation following the established protocols in the lab. Using electrophysiological approaches, I will characterize the synaptic transmission pattern in vitro and in vivo under the impact of lacking or excessive GABA signaling among neurons with or without DISC1 mutation. A detailed understanding of signaling pathways involving DISC1 in regulating synaptic development at the cellular level may provide novel mechanistic insights and lead to new therapeutic strategies.

SIGNALS COOPERATING WITH RUNX1 TO SPECIFY ADULT HSC FROM HESC

Principle Investigator: Yang Song
Institution: Johns Hopkins University
In collaboration with Innovative Biosensors, Inc.
Project Budget : $110, 000

RUNX1 is required for formation of adult, pluripotent hematopoietic stem cells (HSC) during development and for subsequent maturation of HSC to several blood lineages. We are using human embryonic stem cells (hESC) to determine whether exogenous RUNX1 or variants of RUNX1 with increased activity can enable formation of adult HSC from ESC, for clinical application. RUNX1 is encoded from two promoters to yield the RUNX1b and RUNX1c isoforms. Using quantitative RT-PCR we demonstrate that during hESC differentiation, RUNX1b expression peaks first followed by maximal expression of RUNX1c at the time of blood cell formation. We also find that phosphorylation of RUNX1c on serines 48, 303, and 424 increases its activity by reducing interaction with HDAC1 and HDAC3. In an effort to improve typically non-existent generation of adult HSC from hESC, we therefore focus on exogenous expression of RUNX1c(tripleD), in which these three serines are changes to aspartic acid to mimic phosphorylation. Another group found that SHP2-mediated tyrosine dephosphorylation of RUNX1 increases RUNX1 activity, and we find that SHP2 knockdown reduces C/EBPa transcription and that this effect is rescued by exogenous RUNX1. Thus, during granulopoiesis SHP2 inactivates RUNX1 to stimulate C/EBPa transcription. SHP2 may similarly activate RUNX1 during adult HSC formation downstream of alternative external cues. Based on these findings, we also intend to express a RUNX1 variant with relevant tyrosine residues changed to phenylalanine in hESC, as an additional effort to optimize adult HSC formation. Generation of adult HSC from hESC or from autologous iPSC would offer a novel approach to therapy of marrow failure syndromes, would provide a source of autologous HSC to cancer patients requiring marrow ablative doses of chemotherapy, and would provide a novel vehicle for gene therapy applications.

SIGNALS COOPERATING WITH RUNX1 TO SPECIFY ADULT HSC FROM HESC

Principle Investigator: Yijing Su
Institution: Johns Hopkins University
Project Budget : $110, 000

DNA demethylation is implicated in numerous biological processes, such as transposable element silencing, genomic imprinting and X chromosome inactivation. In mammalian cells, DNA methylation is predominantly found at CpG sites. During early embryogenesis, CpG methylation is essential for X-inactivation and asymmetric expression of imprinted genes. In some cases, such as in mouse and human embryonic stem (ES) cells, DNA methylation can also be observed in non-CpG contexts, suggesting that different methylation mechanisms are involved in the maintenance of pluripotency in ES cells. While DNA methylation is well known to be mediated by DNA methyltransferases (DNMTs), the molecular mechanisms controlling DNA demethylation have been under debate. Recent studies led to the prediction that three human enzymes Tet1/Tet2/Tet3 catalyze 5-methylcytosine hydroxylation (5hmC). Hydroxymethyl cytosine (5hmC), a novel modification of DNA, has been proposed to be a critical intermediate for DNA demethylation and could profoundly impact our understanding of gene regulation and epigenetic regulation. Recently, 5hmC was identified as an epigenetic marker in a variety of cell types, such as mouse ES cells and Purkinje neurons. While Tet proteins have been implicated in mouse ES cell biology and cancer, the biological function of Tet proteins and 5hmC in human pluripotent stem cells, neuronal stem cells and neurons is largely unknown. I will investigate the functional role of Tet proteins in regulating human ES and induced pluripotent stem cells and their neuronal derivatives using both gain-of-function and loss-of-function approaches. Pluripotent stem cells and the process of inducible differentiation provide a robust and tractable system to investigate how the epigenetic code influences cellular fate. My study should reveal critical new information oncerning the role of Tet proteins, 5hmC, and DNA demethylation in stem cell regulation and potential new strategies to optimize somatic cell reprogramming.

Role of Electrical Stimulation on Differentiation of Human iPS Cells and iPS-Derived Neural Crest Stem Cells

Principle Investigator: Shuming Zhang
Institution: Johns Hopkins University
Project Budget : $110, 000

It has long been recognized that various living tissues have naturally occurring electrical activities. Previous observations on how such electrical activities can influence tissue re-modeling, cells migration, proliferation and differentiation raises the prospect that electrical stimulation can be an effective cue for manipulating stem cells proliferation and differentiation. To test this hypothesis, we cultured human embryonic stem cell-derived neural crest stem cells (hNCSCs) on a custom-designed platform where various electrical parameters (field polarity, intensity, pulse time and frequency) can be varied independently. We first cultured hNCSCs on the cathode (Matrigel-coated gold thin film) under a 1-Hz, 50-ms electric pulse at a potential gradient of 0, 75, 150 and 200 mV/mm, respectively. Gold anodes were set perpendicular to the substrate to maximize the exposure of cellular bodies to electrical field. After 24 h of stimulation, only cells cultured at a potential gradient of 150 and 200 mV/mm showed significant growth of neurite-like processes. Similar differentiation trend was observed even when the stimulation duration was reduced to 5–16 h, or when NCSCs were subjected to daily 1.5- or 3-h stimulations at 200 mV/mm for three days. The latter stimulation protocol triggered much lower level of apoptosis. Interestingly, when the electrical potential polarity was switched by culturing these cells on the anode, no significant morphological changes were observed. These results support our hypothesis that such electric field can influence stem cells differentiation by mediating ion influx through cell membrane. As calcium signaling is involved in many cellular responses, we further hypothesized that our electrical stimulation protocol increases calcium ion influx, which in turn mediates the intracellular signaling and preferential differentiation of hNCSCs towards neural lineages. Using Fluo4 dye to monitor calcium level changes under the same electrical stimulation conditions described above, we showed that cells cultured on cathodes have much higher and persistent levels of intracellular calcium ions than those cultured with no stimulation or those cultured on the anode. Similar results were obtained for human ESC-derived neural stem cells under these stimulation conditions. Our results demonstrated that such electric stimulation is an effective means to promote neuronal differentiation of human ESC-derived neural stem cells. This new platform may offer a unique approach to probe calcium-mediated intracellular signaling relevant to fate specification and preferential differentiation.

Maryland Stem Cell Research Fund

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