Annual Report on the Nebraska Stem Cell Research Act (LB 606) (Neb.Rev.Stat. §71-8801 et seq)

Presented to the State of Nebraska Legislature

Nebraska Stem Cell Research Advisory Committee and the Nebraska Department of Health and Human Services

March 26, 2012

Introduction

The Nebraska Stem Cell Research Act (LB 606) was passed in the 2008 Legislative Session. The cite reference is Neb.Rev.Stat. §71-8801 et seq.

Stem Cell Research Advisory Committee

This Act created the Stem Cell Research Advisory Committee. Members include the dean of each medical school in Nebraska accredited by the Liaison Committee on Medical Education (Creighton University and the University of Nebraska Medical Center), or his/her designee. In addition, four scientists from outside Nebraska were approved by the 2009 Legislature as members of the Advisory Committee. These individuals are currently being considered for reappointment by the Legislature. The current membership of the Stem Cell Research Advisory Committee includes:

- Bradley Britigan, M.D., University of Nebraska Medical Center
- Rowen Zetterman, M.D., Creighton University
- Bradley Keller, M.D., University of Louisville
- Dennis Roop, Ph.D., University of Colorado-Denver
- Gerald Spangrude, Ph.D., University of Utah
- Rebecca Morris, Ph.D., The Hormel Institute at the University of Minnesota

The Committee develops the grant process and making grant awards to Nebraska institutions or researchers to conduct stem cell research that does not use human embryonic stem cells. The Committee is also responsible for submitting a report to the Legislature on the progress of those projects that have been awarded grants.

Eligibility

Grants are awarded as defined below:

- Sponsoring Institution. Proposals for funding may be submitted by an institution in Nebraska that has an ongoing, large-scale research program that is conducive to the completion of a complex project in stem cell research that does not use human embryonic stem cells (e.g., the University of Nebraska-Lincoln, Creighton University, the University of Nebraska Medical Center, and Boys Town National Research Hospital).

- Principal Investigator. The leader of a project is the "principal investigator" (PI). Researchers with a doctoral degree in science (PhD or equivalent), or a professional degree in a medical field (MD, DMD, DVM, or similar), are eligible to submit a proposal to the Stem Cell Research Advisory Committee as a PI. The PI must be employed at an institution in Nebraska that meets the criteria for "Sponsoring Institution" (see above). Researchers that are classified as Post-doctorates or Fellows are not eligible.

Availability of Funds and Matching Requirements

The amount of money available each year is determined by the Legislature. Beginning with the 2012 grant cycle, only one-year grant applications are available. No single institution or researcher is eligible to receive more than 70 percent of the funds available for distribution.

Each Sponsoring Institution or researcher is responsible to provide a dollar-for-dollar match. The matching funds must be obtained from sources other than funds provided by the Stem Cell Research Act (e.g., principal investigator's salary provided by the sponsoring institution, other research grants from federal sources, stipends for students and post-doctorates).

Submission Requirements

Each proposal must be vetted and approved by a local committee appointed by the Sponsoring Institution before it is accepted by the Stem Cell Research Advisory Committee for full review. The composition of the committee is at the discretion of the higher education institution. Approval of the application by the Sponsoring Institution should be based upon the degree to which the proposal appears to meet the selection criteria.

Proposals that are vetted and approved by the Sponsoring Institutions are submitted via their Office of Sponsored Programs to the Division of Public Health of the Nebraska Department of Health and Human Services. Each Sponsoring Institution may submit a maximum of five proposals in a given funding cycle and no Principal Investigator may hold more than a single award.

Stem Cell Grants Awarded

The first Request for Applications was issued May 13, 2009. The Stem Cell Research Advisory Committee reviewed 13 applications and approved six. The total amount funded for these grants was $900,000 (included two years of funding). The following 2009 grants ended June 30, 2011:

1. Dr. Iqbal Ahmad (University of Nebraska Medical Center): "Adult Stem Cells for Autologous Cell Therapy"; awarded a total $150,000 over two years.
2. Dr. Kirk Beisel (Creighton University School of Medicine): "Transcript Factors in Auditory Hair Cell Regeneration"; awarded a total $150,000 over two years.
3. Dr. Andrea Cupp (University of Nebraska-Lincoln): "VEGF Isoform Regulation of Spermatogonial Stem Cells"; awarded a total $150,000 over two years.
4. Dr. A. Angie Rizzino (University of Nebraska Medical Center): "Human iPS Cell Formation and Sox2-Associated Proteins"; awarded a total $150,000 over two years.
5. Dr. Garrett Soukup (Creighton University School of Medicine): "MicroRNA Promotion of Hair Cell Differentiation"; awarded a total $150,000 over two years.

6. Dr. Jialin Zheng (University of Nebraska Medical Center): "Regenerative Therapy of Parkinson's Disease by iPS Cells"; awarded a total $150,000 over two years.

The 2010 Request for Applications was issued February 12th. After reviewing 15 applications, four grants were funded for a total amount of $483,580.

1. Dr. Hesham Basma (University of Nebraska Medical Center): "Induced Pluripotent Stem Cells and COPD"; awarded a total $129,500 over two years.
2. Dr. Shi-Jian Ding (University of Nebraska Medical Center): "Proteomic Study of iPS and NPC Cell Induction"; awarded a total $129,500 over two years.
3. Dr. George Oyler (University of Nebraska-Lincoln): "Tcd Based Protein Delivery of iPSC Reprogramming Factors"; awarded a total $95,080 for one year.
4. Dr. John Sharp (University of Nebraska Medical Center): "Mouse and Human iPS Cells: Tools to Probe Cellular Aging"; awarded a total $129,500 over two years.

The 2011 Request for Applications was issued February 11th. After reviewing 11 applications, four grants were funded, totaling $506,420.

1. Dr. Jung Yul Lim (University of Nebraska-Lincoln): "Controlling Stem Cell Fate via Cell Patterning"; awarded a total of $150,000 over two years.
2. Dr. Mayumi Naramura (University of Nebraska Medical Center): "Regulation of Hematopoietic Stem Cell Homeostasis by CBL"; awarded a total of $150,000 over two years.
3. Dr. Anuradha Subramanian (University of Nebraska-Lincoln): "Mesenchymal Stem Cells, Scaffolds, and Ultrasound in Cartilage Tissue Engineering"; awarded a total of $56,420 for one year.
4. Dr. Zhao-Yi Wang (Creighton University Medical Center): "ER-alpha36: Roles in Breast Cancer Stem Cells"; awarded a total of $150,000 over two years.

Progress Report of Funded Grants

Some of the major highlights for the first three years of the Nebraska Stem Cell Research Project include:

- Applications submitted to the National Institutes of Health (NIH) and the AO Foundation, leveraging over $4 million in funds.

- Fifteen articles and two book chapters have been published. One article also was reflected on the journal's cover. Nine articles and/or manuscripts are currently being prepared for publication.

- Sixteen research positions have been created (e.g., graduate assistants, post-doctoral research positions).

- Twenty-eight national and/or international presentations relating to funding from the Nebraska stem cell research project have been presented.

Summary of 2009 Funded Grants

Below is a summary of the stem cell grants that began July 1, 2009 and ended June 30, 2011. The information was provided by the Principal Investigator.

Dr. Iqbal Ahmad (UNMC): "Adult Stem Cells for Autologous Cell Therapy": *Stem cell approach holds promise to treat intractable neurodegenerative changes that lead to blindness in age-related macular degeneration (AMD) and retinitis pigmentosa (RP) by cell replacement. However, the approach remains clinically impractical because of two significant barriers: the lack of accessible, renewable and ethically acceptable sources of cells and the possibility of immune rejection. Our goal is to characterize adult stem cells that regenerate cornea as a renewable source of retinal progenitors from which photoreceptors and their precursors can be derived for stem cell therapy.*

Dr. Kirk Beisel (Creighton University): "Transcript Factors in Auditory Hair Cell Regeneration": *Hair cell loss in the inner ear is a major cause of deafness. Currently, no therapies exist for hair cell regeneration, but factors that influence normal hair cell development might be used to reprogram stem cells. The goal of the project was to establish whether a combination of transcription factors (Atoh1, Pou4f3 and Gfi1) influences cellular gene expression to become more hair cell-like. The project has progressed to development of vectors for delivery and assessment of effects on stem cells and is combined with Dr. Soukup's deliver of microRNAs in combination with our three transcription factors.*

Dr. Andrea Cupp (University of Nebraska-Lincoln): "VEGF Isoform Regulation of Spermatogonial Stem Cells": *We have determined that VEGFA isoforms do regulate the spermatogonial stem cell (SCC) niche. VEGFA164 (angiogenic isoform) causes increased colonization of stem cells in testes while VEGFA165b (antiangiogenic isoforms) reduced colonization and presumably cause differentiation or apoptosis. (Cover picture on Endocrinology, February issue, 2012; http://endo.endojournals.org) We also have made cell specific mice to determine how the balance of these antiangiogenic and angiogenic VEGFA isoforms affect the stem cell niche.*

Dr. A. Angie Rizzino (UNMC): "Human iPS Cell Formation and Sox2-Associated Proteins": *Fibroblasts and pluripotent stem cells were engineered to express from an inducible promoter different combinations of reprogramming factors, including the transcription factor Sox2, when the cells are treated with a small molecule inducer. These cells were used to perform an unbiased proteomic screen to identify proteins that associate with Sox2 under different cellular contexts. This study has provided new insights into the molecular mechanisms needed to reprogram somatic cells to induced pluripotent stem cells.*

Dr. Garrett Soukup (Creighton University): "MicroRNA Promotion of Hair Cell Differentiation": *Hair cell loss in the inner ear is a major cause of deafness. Currently, no therapies exist for hair cell regeneration, but factors that influence normal hair cell development might be used to reprogram stem cells. The goal of the project was to establish whether a combination of such factors influences cellular gene expression to become more hair cell-like. The project has progressed to development of vectors for delivery and assessment of effects on stem cells.*

Dr. Jialin Zheng (UNMC): "Regenerative Therapy of Parkinson's Disease by iPS Cells": *This project proposed to develop induced pluripotent stem (iPS) cells from brain astrocytes and address the potential of using iPS cells as a therapeutic strategy to treat an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD). In past two years, we have successfully generated astrocyte-derived iPS cells and further found that reprogrammed astrocytes retain a "memory" of the central nervous system, which confers additional potential upon neuronal differentiation. These findings provide strong support for astrocyte-derived iPSCs as a therapeutic strategy to treat an MPTP mouse model of PD.*

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Dr. Hesham Basma (UNMC): "Induced Pluripotent Stem Cells and COPD": *Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease of the lung. Recent studies have shown that fibroblasts isolated from COPD patients have abnormal repair functions compared to fibroblasts of matched controls. Altered repair is believed to contribute to the progressive alteration of lung structure. Induced pluripotent stem cells (iPSCs) have the potential to advance the therapeutic strategy of restoring lung function. The current study is testing the hypothesis that the abnormal phenotype of COPD fibroblasts can be corrected by reprogramming through the formation of (iPSCs).*

Dr. Shi-Jian Ding (UNMC): "Proteomic Study of iPS and NPC Cell Induction": *We proposed to develop and apply quantitative proteomics technology to investigate the key intracellular signaling pathways and protein targets that control the development of induced pluripotent stem cells (iPSCs) and their progression into neural progenitor cells (NPCs) from somatic cells. The NPCs offer stem cell-based reparative approach for neurotrauma and neurodegenerative diseases, afflicting over ten million people worldwide.*

Dr. George Oyler (University of Nebraska-Lincoln): "Tcd Based Protein Delivery of iPSC Reprogramming Factors": *This project examines the potential to reprogram somatic cells into induced pluripotent stem cells (iPSC) using protein delivery of factors rather than genetic modifications of the cells. Such protein based reprograming is desirable because it avoids the risks associated with genetic modifications in the reprogramming process. The strategy to achieve protein-based delivery of the reprograming factors involves the harnessing portions of bacterial toxin proteins to deliver a desired cargo of the stem cell factors. This research has far*

reaching possibilities for intracellular protein delivery in the stem cell arena and in other aspects of human medicine ranging from treating neurological diseases to cancer therapies.

Dr. John Sharp (UNMC): "Mouse and Human iPS Cells: Tools to Probe Cellular Aging": *Adult tissue cells can be reprogrammed to express stem cell characteristics (induced pluripotent cells or IP cells). IP cells are immunologically matched to the donor and have the potential to repair or regenerate damaged tissues. Patients who are likely to need tissue repair are generally elderly. This project addresses the question as to whether "old" cells can be reprogrammed as easily as "young" cells.*

The research shows that "old" adult bone marrow cells do not reprogram as easily as young mesenchymal stromal (MEF) cells. In addition, adult hematopoietic stem cells, rather than non-stem cells have to be used. Furthermore, they have to be grown on supporting stromal cells to prevent them from dying during reprogramming. As stem cells age, they accumulate DNA damage. A protein (P16ink4a) is produced that stops these cells from proliferating because the damage could lead to tumor formation. We are now testing whether the presence of this protein is why "old" cells are more difficult to reprogram than "young" cells.

In collaboration with Iqbal Ahmad, Ph.D., we have tested if reprogrammed cells form tumors. They do form teratomas in immunodeficient mice. Consequently, the use of IP cells for tissue repair, especially in the elderly, is likely to be much more difficult than originally believed.

Summary of 2011 Funded Grants

Below is a summary of the stem cell grants that began July 1, 2011 and will end June 30, 2013. Information was provided by the Principal Investigator.

Dr. Jung Yul Lim (University of Nebraska-Lincoln): "Controlling Stem Cell Fate via Cell Patterning": *Cell micropatterning has demonstrated a potential to direct mesenchymal stem cell (MSC) function and fate. We aim to control cell-to-cell interaction, which plays a critical role in cellular lineage commitment and differentiation, by patterning MSCs into interconnected or isolated geometry as a novel way of controlling stem cell fate. By assessing patterned cell behavior and revealing relevant signaling mechanism, an integrated picture on how to control MSC fate via physical constraints will be provided.*

Dr. Mayumi Naramura (UNMC): "Regulation of Hematopoietic Stem Cell Homeostasis by CBL": *Hematopoietic stem cell transplant (HSCT, also called "bone marrow transplant") is now well established as a curative treatment for various malignant and non-malignant diseases. However, there are needs for further improvement in this life-saving procedure. Based on our preliminary studies in gene targeted mouse models, we identified the Cbl family E3 ubiquitin ligases as critical regulators of HSC homeostasis and formed a hypothesis that temporary and reversible inhibition of Cbl activity will facilitate HSCT. We are currently testing this hypothesis by generating an inducible gene expression knock-down system and by examining potential Cbl*

targets in the HSC compartment to better understand how Cbl proteins regulate HSC homeostasis.

Dr. Anuradha Subramanian (University of Nebraska-Lincoln): "Mesenchymal Stem Cells, Scaffolds, and Ultrasound in Cartilage Tissue Engineering": *To optimize an engineered microenvironment to guide MSC differentiation to chondrocytes using US, 3D scaffolds and growth factors. (This grant ends June 30, 2012.)*

Dr. Zhao-Yi Wang (Creighton University Medical Center): "ER-alpha36: Roles in Breast Cancer Stem Cells": *Novel mechanisms and targets for development of effective therapeutic approaches to eliminate breast cancer stem/progenitor cells are urgently needed. In this project, we study the biological function and underlying mechanisms of a novel estrogen receptor, ER-α36 and its signaling in ER-positive and –negative breast cancer stem/progenitor cells. We also directly explore the possibility of using ER-α36 as a novel target to eradicate breast cancer stem/progenitor cells.*

Conclusions

Although the Nebraska Stem Cell Research Project is relatively new, substantial progress has been made and a solid stem cell research foundation has been established. The stem cell research grants may lead to innovative medical treatment options for diseases such as blindness in age-related macular degeneration, hearing loss, breast cancer, and Parkinson's disease. In addition, this program has created new positions, multiple articles and publications have been submitted to research journals, and presentations have occurred at national and international meetings. Finally, researchers are using their Nebraska stem cell funds as leverage in applying for new grant applications from various institutions.