
# 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 24, 2017**

## Introduction

The Nebraska Stem Cell Research Act (LB 606) was passed in the 2008 Legislative Session (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 School of Medicine and the University of Nebraska Medical Center), or his/her designee. Four scientists from outside Nebraska also serve as members of the Advisory Committee (one current vacancy). The current membership of the Stem Cell Research Advisory Committee includes:

- Bradley Britigan, M.D., Dean, University of Nebraska Medical Center, College of Medicine
- Robert Dunlay, M.D., Dean, Creighton University School of Medicine
- Rebecca Morris, Ph.D., The Hormel Institute at the University of Minnesota
- Alysson Muotri, Ph.D., University of California – San Diego
- Dennis Roop, Ph.D., University of Colorado – Denver

The Committee is responsible for developing the grant process and making recommendations on grants to the Nebraska Chief Medical Officer. Institutions or researchers may not receive stem cell funding if using human embryonic stem cells. The Committee is also responsible for submitting an annual report to the Legislature on the progress of awarded projects.

## Eligibility

Awards are granted as defined below:

- Sponsoring Institution. Preference will be given to funding proposals 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.

- 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. As provided in Neb.Rev.Stat. §71-8805, no single institution or researcher is eligible to receive more than 70 percent of the funds available for distribution.

Each Sponsoring Institution or researcher must provide a dollar-for-dollar match. See Neb.Rev.Stat. §71-8805. 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, or its equivalent, before it is accepted by the Stem Cell Research Advisory Committee for full review. 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 local committee or its equivalent, must be submitted 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.

### 2016 Stem Cell Grants

After reviewing 11 applications, four grants were funded, totaling $435,000. These grants will end June 30, 2017. The summaries were provided by the Principal Investigators.

*Shannon Buckley, PhD (University of Nebraska Medical Center): Role of E3 Ubiquitin Ligase, FBX08, in Maintaining & Inducing Pluripotency*; received $109,468 for one year

*Project Summary*: Pluripotent stem cells (embryonic stem cells; ESC and induced pluripotent stem cells; iPSC) have the unique characteristics that they can differentiate to all three germ layers and are capable of indefinite self-renewal. Our previous studies suggested that key elements of the pluripotency network (including the "master" regulators Nanog and Oct4) are controlled by ubiquitination and that a significant number of UPS members (mainly ubiquitin E3 ligases) regulate pluripotency and influence lineage differentiation. Within the E3 ligase family, FBX08 is an intriguing ligase to propose for further study for a number of reasons: 1) very little is known about FBX08; 2) loss of FBX08 enhances efficiency of cellular reprogramming; 3) it has recently been shown that E3 ligases, including F-box proteins represent a class of proteins that are "druggable" targets due to structure and number of downstream substrates. The goal of our studies is to understand the role of E3 ligase FBX08 and its substrates in cellular reprogramming to achieve optimal cell reprogramming and accelerate clinical applications of induced pluripotent stem cells.

*Xian-Ming Chen, MD (Creighton University): Intestinal Stem Cell Responses to C. Parvum Infection*; received $110,000 for one year

*Project Summary*: With a long-term goal to better understand the molecular mechanisms of gastrointestinal diseases for more efficient therapeutic interventions, this application aims to investigate the responses of distinct intestinal stem cells (ISCs) to pathogen infection at the intestinal mucosal surface, using models of intestinal infection by *Cryptosporidium parvum* (*C.*

*parvum*), a protozoan parasite that usually only infects epithelial cells (enterocytes) at the villus tip.

*Andrea Cupp, PhD (University of Nebraska – Lincoln): Purification & Proliferation of Mouse Spermatogonial Stem Cells*; received $110,000 for one year

*Project Summary*: Few markers distinguish Spermatogonial Stem Cells (SSCs) from other male germ cells. Additionally, SSCs require a feeder layer when cultured, and spermatogonial stem cell transplantation (SSCT: transplanting donor germ cells into endogenously depleted germ cell recipients and determining colonization efficiency) is the only assay to determine SSCs. Our long term objective is to develop combinations of markers that identify the SSC population and a culture system to allow for increased proliferation of these rare cells from adult testes in vitro. Thus, our hypothesis is that a specific combination of proteins will define a more enriched population of SSCs that can be increased through in vitro proliferation with growth factor combinations added to a 3-D alginate culture system. The aims to test this hypothesis are: 1) evaluate combinations of protein antibodies: ID4, PAX7, TSPAN8 and NRP1 for a purified SSCs through cell sorting and flow cytometry followed by SSCT to determine colonization efficiency and 2) determine combinations of growth factors (GDNF, FGF) that could be utilized in a 3-D alginate culture system followed by SSCT to identify greater proliferation of SSCs.

*Haitao Wen, PhD (University of Nebraska Medical Center): O-GlcNAc Signaling in Intestinal Stem Cell Mediated Epithelial Regeneration*; received $105,532 for one year

*Project Summary*: The intestinal epithelium is the most rapidly self-renewing tissue in the mammalian body and its process is controlled by the intestinal stem cells (ISCs). This project is to investigate the role of the *O*-GlcNAc signaling in ISC-mediated epithelial regeneration following sepsis-induced intestinal tissue damage.

## 2015 Stem Cell Grants

After reviewing ten applications, five grants were each funded for $87,400 (for one year), totaling $437,000. These grants ended June 30, 2016. The summaries were provided by the Principal Investigators.

*Andrea Cupp, PhD (University of Nebraska – Lincoln): Mechanisms of VEGFA Isoforms on Germ Stem Cells*

*Project Summary:* Recent studies from our laboratory have identified a role for vascular endothelial growth factor A (VEGFA) in fertility and, in Spermatogonial Stem Cell maintenance. Interestingly, the *Vegfa* gene can be spliced into either angiogenic or antiangiogenic isoforms. Cell-specific elimination of VEGFA from the testis has resulted in reduced male fertility and altered expression of genes known to regulate SSCs. Treatment with the antiangiogenic isoform, VEGFA165b, in male mice has been shown to reduce SSC colonization in recipients following transplantation of donor germ cells. Thus, we hypothesize that VEGFA angiogenic isoforms are critical for SSC proliferation and that VEGFA antiangiogenic isoforms reduce SSC numbers by initiating cell death pathways. Two aims will test this hypothesis: Aim #1: Determine the direct effects of VEGFA angiogenic isoforms on SSC proliferation; Aim #2: Determine the potential

mechanisms of SSC reduction induced by VEGFA antiangiogenic isoform signaling in male germ cells. A better understanding of the mechanisms of how VEGFA angiogenic and antiangiogenic isoforms regulate SSC maintenance could allow for development of therapeutic strategies in infertile males, potential contraceptive methods and a better understanding of stem cell biology in general.

*R. Katherine Hyde, PhD (University of Nebraska Medical Center): The Role of RUNX1 and GATA2 in Leukemia Stem Cells*

*Project Summary:* Leukemia is a cancer of the immature blood cells in the bone marrow. Patients with leukemia often relapse because traditional treatments don't effectively kill leukemia stem cells (LSCs); the small population of leukemia cells maintain the disease. In this project, we are investigating two proteins, RUNX1 and GATA2 that are known to be important in normal hematopoietic stem cells to determine if they play similar roles in LSCs.

*Yuguo Lei, PhD (University of Nebraska – Lincoln): Novel Stem Cell Therapy for Parkinson's Disease*

*Project Summary:* In this project, we aim to develop a fibrin device with a cocktail of pro-survival factors for simultaneously delivering dopaminergic (DA) neurons and creating a pro-survival therapeutic niche to enhance the survival of delivered DA neurons for treating Parkinson's disease (PD). In the proposed device, engineered fibrin hydrogel will carry cells and pro-survival factors encapsulated in Poly lactic-co-glycolic acid (PLGA) microspheres. The hydrogel will support cell growth and the PLGA microspheres will temporally release the factors. The cocktail will contain factors that can suppress all known mechanisms that cause cell death. It will simultaneously suppress inflammation, promote angiogenesis, suppress excitotoxicity, scavenge reactive oxidative species, and inhibit apoptosis.

*Jung Lim, PhD (University of Nebraska – Lincoln): Mechanical Stretch Control of Stem Cell Fate*

*Project Summary:* This project aimed to determine the role of N-cadherin cell-cell adherens junction in mechanical stretch-induced mesenchymal stem cell (MSC) linage commitment toward osteogenesis vs. adipogenesis. We observed that mechanical stretch induces the disassembly of β-catenin from N-cadherin junction and directs β-catenin nuclear translocation, thus improving stem cell osteogenic transcription. Our results suggest the role of N-cadherin junction and related mechanosensor, β-catenin, in controlling MSC fate.

*A. Angie Rizzino, PhD (University of Nebraska Medical Center): Cancer Stem Cells of Pancreatic Ductal Adenocarcinoma*

*Project Summary:* The main goals of this work were to determine whether altering the levels of the stem transcription factor SOX2 influences the frequency of pancreatic cancer tumor-initiating cells and the responses of these cells to drugs used in clinical trials to treat this highly deadly cancer.

## Conclusions

The Nebraska Stem Cell Research Project has shown substantial progress and a solid stem cell research foundation has been established. Research has included gastrointestinal diseases, Parkinson's Disease, chronic obstructive pulmonary disease (COPD), breast cancer, reparative therapies for individuals who suffer from neurotrauma and neurodegenerative diseases, retina repair, clinical alternatives to creating replacements for restoring normal bone tissues, male fertility, leukemia, and sepsis-induced intestinal tissue damage.

Researchers have used their Nebraska stem cell funds as leverage in applying for new grant applications from agencies such as the National Institutes of Health (NIH), American Cancer Society, Leukemia & Lymphoma Society, and the Bill Gates Foundation.

## Progress Report of Funded Grants

Some of the major highlights of the Nebraska Stem Cell Research Project during 2015-2017:

- Nebraska researchers have received additional funds from NIH that were directly related to their project. Pending submissions are approximately $100,000.
- 15 publications (i.e., articles, manuscripts, papers) have been published or are under consideration for publication.
- Approximately 15 research positions resulted from these grants (full and/or part-time)
- Approximately 20 national and/or international presentations relating to funding from the Nebraska Stem Cell Research Project have been presented.