# NEBRASKA

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### **DEPT. OF HEALTH AND HUMAN SERVICES**

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, 2019

#### 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. 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
- Rui Yi, Ph.D., University of Colorado Boulder

The Committee is responsible for developing the grant process and making recommendations on grants to the Division of Public Health 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:

- <u>Sponsoring Institution</u>. 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.
- <u>Principal Investigator</u>. 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.

#### 2018 Stem Cell Grants

After reviewing eight applications, five grants were funded, totaling \$436,500. These grants will end June 30, 2019. The summaries were provided by the Principal Investigators.

Iqbal Ahmad, PhD (University of Nebraska Medical Center): Human Disease Modeling of Glaucoma; received \$101,850 for one year

*Project Summary*: Glaucoma is the second leading cause of blindness in the world, which is due to the degeneration of specific retinal neurons-the retinal ganglion cells (RGCs)-that connect with the higher centers in the brain for visual perception. We are generating RGCs from glaucoma patients and studying these cells in controlled conditions to understand the cause of the degeneration and formulate approaches for therapeutic intervention.

*Matthew Dilisio, MD (Creighton University): Stem Cell Derived Exosome Delivery for Tendon Repair, received \$20,950 for one year* 

*Project Summary*: The goal of our work is to harness the regenerative capacity of mesenchymal stem cells in order to provide better treatment modalities for patients suffering rotator cuff injuries. We aim to synthesize a hydrogel system [(PVA-Alginate-

PPF (PAP)] by the interpenetration and subsequent cross linking of the natural polysaccharide alginate to optimize the biologic availability and half-life of the stem cell exosomes to interact with host tissues.

Sung-Ho Huh, PhD (University of Nebraska Medical Center): Mechanism Regulating Sensory Stem Cell Generation; received \$101,850 for one year

*Project Summary*: This project is to understand the role of Wnt/beta-catenin signal in auditory sensory stem cells. Auditory epithelia, which are responsible to hearing, are not able to regenerate after damage, resulting in permanent hearing loss. By understanding mechanisms of auditory stem cell generation and maintenance, we may develop a new strategy to regenerate damaged auditory epithelium, promoting ultimate hearing restoration.

Jung Yul Lim, PhD (University of Nebraska – Lincoln): Role of YAP Mechanotransduction in MSC Fate Decision; received \$110,000 for one year

*Project Summary*: The specific aim of this study is to determine the role of Yes-Associated Protein (YAP) in regulating mechanosensitive mesenchymal stem cell (MSC) fate decision. Specifically, we test the hypothesis that stretch loading-induced activation of YAP signaling pathway is responsible for MSC lineage specification to osteogenesis. We further examine the potential crosstalk between YAP stretch mechanotransduction and cytoskeleton-nucleus force transfer in MSCs.

Jingwei Xie, PhD (University of Nebraska Medical Center): ADSCs-seeded 3D Aerogels for Bone Regeneration; received \$101,850 for one year

*Project Summary*: We have been working on this project. We have published three papers supported by this grant. We have also submitted another manuscript supported by this grant. Based on this project, we are preparing an administrative supplements application to NIH NIDCR. In addition, based on this project, we have submitted an NIH R21 grant.

#### 2017 Stem Cell Grants

After reviewing 11 applications, four grants were funded, totaling \$414,500. These grants ended June 30, 2018. The summaries were provided by the Principal Investigators.

Bin Duan, PhD (University of Nebraska Medical Center): MSC Derived Myelinating Schwann Cell for PN Regeneration; received \$101,500 for one year

*Project Summary*: We have successfully and repeatedly induced human adipose derived mesenchymal stromal cells (hADMSC) into schwann cell (SC) like cells on our novel nanofiber yarns. We have confirmed the culture condition to improve the myelination properties of hADMSC-SC on both 2D culture and nanofiber yarns. We have implanted the conduits with nanofiber yarns into a rat model with peripheral nerve defect and

demonstrated that the implementation of nanofiber yarns could improve peripheral nerve regeneration.

Anna Dunaevsky, PhD (University of Nebraska Medical Center): Modeling Fragile X Syndrome with Stem Cells; received \$101,500 for one year

*Project Summary*: The goal of the project was to establish a human cell based model for Fragile X syndrome, a neurodevelopmental disorder, using induced pluripotent stem cells (iPSCs). We have been studying astrocytes, non-neuronal brain cells, differentiated from FXS and control patient iPSCs in culture and *in vivo* following engraftment in mice. We have already discovered several impairments in the FXS cells including altered cell cycle, altered maturation pattern and impaired functional (calcium) signaling. This model will continue to provide important insights into the role of FMRP in astrocytes to disease pathology, progression, and mechanism.

#### Yuguo Lei, PhD (University of Nebraska – Lincoln): A Method for Culturing Induced Pluripotent Stem Cells; received \$110,000 for one year

*Project Summary*: Specific Aim 1 is to systematically study how the parameters of AlgTubes including the alginate hydrogel concentration, tube diameter, hydrogel shell thickness and initial cell seeding density influence iPSC viability, growth, yield and pluripotency. Specific Aim 2 is to systematically compare culturing iPSCs in AlgTubes and the current 2D and 3D suspension culturing.

Jingwei Xie, PhD (University of Nebraska Medical Center): 3D Scaffolds Homing Stem Cells for Cartilage Repair, received \$101,500 for one year

*Project Summary*: We have obtained some preliminary data for this project. Based on these observations, the cartilage regeneration is not satisfactory. We are planning new experiments to improve the results. Hopefully, we can obtain some preliminary data for a NIH grant application in the future.

#### 2016 Stem Cell Grants

After reviewing 11 applications, four grants were funded, totaling \$435,000. These grants ended 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, FBXO8 is an intriguing ligase to propose for

further study for a number of reasons: 1) very little is known about FBXO8; 2) loss of FBXO8 enhances efficiency of cellular reprogramming; and 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 FBXO8 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. 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.

#### Conclusions

The Nebraska Stem Cell Research Project has shown substantial progress, and a solid stem cell research foundation has been established. Research has included glaucoma, rotator cuff injuries, intestinal stem cell regeneration, Parkinson's disease, therapeutic intervention for various gastrointestinal diseases, retina repair, clinical alternatives to creating replacements for restoring normal bone tissues, peripheral nerve regeneration, cartilage regeneration, male fertility, leukemia, developing new strategies to regenerate damaged auditory epithelium promoting ultimate hearing restoration, genetic bone disorders, studying the pathogenesis of neurodevelopmental diseases, and sepsis-induced intestinal tissue damage.

Researchers have also used their Nebraska stem cell funds as leverage in applying for new grant applications from the National Institutes of Health (NIH), National Science Foundation (NSF), Centers of Biomedical Research Excellence (CoBRE), and Nebraska Center for Nanomedicine (NCN).

#### **Progress Report of Funded Grants**

Some of the major highlights of the Nebraska Stem Cell Research Project during 2016-2019:

- Nebraska researchers have received additional funds exceeding \$2.4 million from NIH, NSF, CoBRE, and NCN that were directly related to their project
- Nebraska researchers have multiple NIH proposals pending/under review
- 28 publications (i.e., abstracts, articles, manuscripts, papers) have been prepared, published, or are under consideration for publication
- 15 research positions resulted from these grants (full and/or part-time)
- 31 national and international presentations relating to funding from the Nebraska Stem Cell Research Project were presented
- 1 new invention notification under preparation; two pending patents
- 1 new regional collaboration established with the Waisman Center to study FXS using human derived stem cells: https://www.waisman.wisc.edu/event/fragile-x-exchange-2019