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

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

March 26, 2016

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:

- Joyce Bischoff, Ph.D., Boston Children's Hospital/Harvard Medical School
- 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
- Dennis Roop, Ph.D., University of Colorado-Denver

The Committee is responsible for developing the grant process and making recommendations on subawards 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

Subawards 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.

2015 Stem Cell Subawards

After reviewing ten applications, five subawards were each funded for $87,400 (for one year), totaling $437,000. These subawards will end 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: Men with low sperm counts represent 40 percent of the population trying to have a family in the US. A potential problem with these men may be less sperm stem cells due to reduced proliferation or increased differentiation or programed cell death. Our project is trying to understand how sperm stem cells proliferate, differentiate and go through programmed cell death. We are investigating a gene-Vascular Endothelial Growth Factor A (VEGFA) that can be processed to produce different isoforms which stimulate stem cell renewal and cause apoptosis of differentiating sperm. We are trying to determine how these gene products interact with other factors to manipulate spermatogenesis and increase sperm production. Our long term goal would be to transition this increased understanding of how different VEGFA isoforms affect sperm stem cells to biomedical and clinical situations of male infertility.

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, which 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: While mechanical stretch loading has demonstrated its potential to direct human mesenchymal stem cell (hMSC) fate, very little is revealed regarding how hMSCs "socially" sense and respond to stretch. This study determines the role of N-cadherin cell-cell adherens junction and related molecular mechanosensor, β-catenin, in stretch-induced hMSC fate decision toward osteogenesis vs. adipogenesis.

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

Project Summary: The goal of this ongoing project is to determine whether elevating SOX2 in pancreatic ductal adenocarcinoma cells and/or treatment of these cells with small molecular inhibitors in vitro alters the number of cancer stem cells in the population of tumor cells. Thus far, we have determined that elevating SOX2 in pancreatic ductal adenocarcinoma cells substantially reduces tumor growth in an animal model. We are in the process of testing whether this is also true when pancreatic ductal adenocarcinoma cells are treated with small molecule inhibitors.

2014 Stem Cell Subawards

After reviewing nine applications, four subawards were funded, totaling $435,986. These subawards ended June 30, 2015. The summaries were provided by the Principal Investigators.

Shashank Dravid, DVM, PhD (Creighton University School of Medicine): mTOR Pathway and Glutamate Delta-1 in Neural Stem Cells; received $110,000 for one year

Project Summary: Fundamental roles of neural stem cells (NSCs) in dentate gyrus and mTOR signaling have been identified in regulating social behaviors relevant to mental disorders particularly autism spectrum disorders. Our data indicate that glutamate delta-1 receptor can regulate NSC proliferation and mTOR signaling and thereby may serve as a therapeutic target in conditions where NSC/mTOR dysfunction may underlie social deficits in mental disorders.

Andrew Dudley, PhD (University of Nebraska Medical Center) and Shadi Othman, PhD (University of Nebraska-Lincoln): Calcium-Optimized Cartilage Formation from MSCs; received $110,000 for one year

Project Summary: The objective of this project is to determine if changing the calcium concentration in the cell culture medium will improve tissue engineering of cartilage from human mesenchymal stem cells (hMSCs). hMSCs respond to changes in calcium concentration that would predict the formation of better cartilage. However, we have not been able to prevent the negative effect of elevated calcium in the form of mineral deposition in the cell layers. We are currently exploring alternative methods to modulate calcium signaling in hMSCs that does not require using elevated calcium concentration.

Stephen Rennard, MD (University of Nebraska Medical Center): Reprogramming Airway Fibroblasts in Asthma; received $105,986 for one year

Project Summary: The proposal was designed to explore the hypothesis that reprogramming of airway fibroblasts is a therapeutic option to alter the natural history of asthma. We will pursue two independent specific aims: Aim #1: Determine if the altered function of asthmatic airway fibroblasts can be "corrected" by epigenetic reprogramming by de-differentiation into iPSCs followed by re-differentiation into fibroblasts in vitro. Aim #2: Identify a defined and limited set of factors that directly trans-differentiate "asthmatic" fibroblasts into "normal" fibroblasts.

A. Angie Rizzino, PhD (University of Nebraska Medical Center): Reprogramming Human Somatic Cells without Transgenes; received $110,000 for one year

Project Summary: The goal of this project was to test the hypothesis that small molecules that precociously activate the endogenous OCT4 gene will enable the reprogramming of human somatic cells into human induced pluripotent stem cells (hIPSC) without the aid of transgenes. We did not identify a cocktail of small molecule inhibitors that could replace the requirement for OCT4 during reprogramming. However, our studies led us to suspect that lowering SOX2 levels in pluripotent stem cells will reduce the heterogeneity in the pluripotent stem cell population.

2009 – 2013 Stem Cell Subawards

During 2009 – 2013, 60 applications were received for the Nebraska Stem Cell Research Project and 23 subawards were funded, totaling $2,778,093. All of these subawards have ended. For this annual report, the Principal Investigators involved in these funded projects were contacted for an update on their research and their information is below.

2013 Stem Cell Subawards

Iqbal Ahmad, PhD (University of Nebraska Medical Center): Therapeutic Regeneration in Diseased Retina

Goals/Aims: The main goal was to unlock the neurogenic potential of Muller glia, the support cell within adult retina with stem cell potential, for therapeutic regeneration in blinding diseases. Two specific aims were proposed to achieve the goal: (1) To determine the mechanism that

inhibits the neurogenic potential of MG. We hypothesized that REST, a global repressor of neuronal genes, and Sox9, a regulator of the neurogenic to gliogenic transition, were both positively regulated by Notch signaling in MG, suppressing neurogenesis. (2) To determine the mechanism that promotes the neurogenic potential of MG. We hypothesized that this was orchestrated by *Lin28*, a heterochronic gene involved in the timing of neurogenesis, in conjunction with *Mash1(Ascl1)*, a pro-neural gene.

Results: We demonstrated that REST is one of the main inhibitors of neurogenic potential, as REST loss of function led to the acquisition of neurogenic properties by enriched Muller glia (Zheng et al., ARVO Abst. 2015). We observed in support of the second specific aim that the acquisition of neurogenic properties was preceded by the expression of *Lin28* and *Mash1* (*Ascl1*) and that *Lin28/Mash1(Ascl1)* gain of function alone could confer neuronal properties on enriched Muller glia (Mir et al., ARVO Abst. 2015.)

Hamid Band, PhD (University of Nebraska Medical Center): Genetic Dissection of Intestinal Crypt Stem Cell Regulation by Cbl-family Ubiquitin Ligases

Goals/Aims: This project investigates a new mechanism, mediated by two members of the CBL-family of ubiquitin ligase proteins, to regulate the renewal of stem cells that maintain the integrity of body tissues. Using unique mouse models, in which genes for CBL proteins are ablated under controlled conditions, we have obtained evidence for a critical requirement of the Cbl protein family in the hematopoietic, mammary and intestinal stem cell renewal. Current studies are geared to extend these findings to address specific disease states and issues in regenerative medicine.

Results: We established a requirement of CBL and CBL-B in hematopoietic, mammary and intestinal stem cell quiescence, which helps them to last long. CBL/CBL-B deficiency expands specific progeny, which may be desirable in certain cases following transplant of stem cells. In the intestine, we have found that CBL proteins control the rate at which stem cells progress to specific mature epithelial lineages such as Goblet cells (which protect the intestine by musus secretion) and Paneth cells (which support stem cell maintenance). The mouse models characterized with the support of this grant have opened up new initiatives in regenerative medicine focused on targeting of CBL proteins to protect against muscle wasting in diseases and aging, and to counter insulin resistance in diabetes, as well as in cancer.

Janee Gelineau-van Waes, DVM, PhD (Creighton University School of Medicine): CerS1-Gdf1: Bicistronic Balance of Neural Stem Cell Fate

Goals/Aims: This project evaluates the role of CerS1 (*Ceramide Synthase 1*) and *Gdf1* (*Growth and differentiation factor 1*), a member of the TGFβ superfamily, in regulating the balance between self-renewal and differentiation of neural progenitor cells through modulation of nuclear vs. cytoplasmic levels of sphingolipid metabolites.

Results: The results of this research project demonstrate that Gdf1 signaling is involved in feedback regulation of CerS1 expression. Inhibition of CerS1 results in preferential accumulation of sphinganine-1-phosphate (Sa1P) in the nuclear compartment of neural progenitor cells. Elevated nuclear Sa1P is associated with inhibition of HDAC (histone deacetylase) activity, and

increased acetylation of specific lysine residues on histones H3 and H4. These epigenetic changes result in altered regulation/expression of genes involved in modulating the balance between self-renewal and differentiation.

Shadi Othman, PhD (University of Nebraska – Lincoln): Multi-Modal Imaging for Bone TE in a Defect Model

Goals/Aims: Develop noninvasive multi-modal imaging for bone tissue engineering that will ultimately replace invasive histology and molecular testing.

Results: We developed an MR compatible bioreactor for in vitro testing and applied multi-model imaging for in vivo animal testing including MRI and optical imaging.

2012 Stem Cell Subawards

Andrea Cupp, PhD (University of Nebraska – Lincoln): VEGFA Isoform Effects on Spermatogonial Stem Cell Homeostatis

Goals/Aims: Spermatogonial stem cells (SSC) are germ line stem cells in the male and upon differentiation they form sperm. Formation of SSC occurs around postnatal day 8-12 and they develop the ability to self-renew at postnatal day 42 in the mouse. Very little is understood about what genes regulate SSC renewal and differentiation. Thus, the hypothesis is that an appropriate balance of pro and antiangiogenic VEGFA isoforms are necessary for the establishment and maintenance of the SSC niche. Two specific aims will use cell-specific conditional knockout mice to: 1) Determine the effects of increased actions of VEGFA antiangiogenic isoforms in Sertoli cells on SSC colonization and proliferation; 2) Investigate the effects of removal of all VEGFA isoforms in Sertoli cells on SSC colonization and proliferation.

Results: The results of this project were that loss of VEGFA isoforms (all) in Sertoli and germ had dramatic effects on the regulation of spermatogonial stem cell proliferation. We also developed other conditional KO lines using a Cre promoter that only was in Sertoli cells- Sry-cre. With this we hoped to demonstrate that knocking out the VEGFA isoforms only in Sertoli was really what was causing the infertility and potential effects on the SSCs. Because we had to have certain amount of females that were conditional KO's to develop these lines of males we also investigated fertility in these females.

Shi-Jian Ding, PhD (University of Nebraska Medical Center): Proteomic Study of iPS and NPC Cell Induction

Goals/Aims: Aim 1: To determine the abundance-profiles of proteins from Astrocytes (ASs), AS-derived iPSCs and NPCs as well as from hepatocytes (HPCs), HPC-derived iPSCs and NPCs. We are still working on Aim 1, which is to apply the mass spectrometry-based proteomic approache to investigate the proteome difference between AS and AS-derived iPS cells, and between iPS cells and iPS-derived NPC cells. Aim 2: To explore the functional significance of O-GlcNAcylation on SOX2 during iPSC and NPC generation. Through our proteomic study, we discovered this novel modification on SOX2. However, six months after we submitted our proposal, Jiang et al., reported that the core reprogramming factors Oct4 and Sox2 are O-

GlcNAcylated in ESCs/iPSCs, but the O-GlcNAc modification is rapidly removed upon differentiation (Jiang et al., Cell Stem Cell 11, 1–13, July 6, 2012). Since the proposed studies in this aim were largely accomplished by the paper, we decided to investigate the histone modification changes during iPSC and NPC generation using our unique UNiquant program and ISPTM approach. We have generated mass spectrometry data and are in the stage of data analysis.

Results: Based on the literature search and in-depth data analysis, we have prioritized several proteins which are significantly upregulated in MEF derived iPS cells comparing to MEF cells. Among these proteins, PP2A is a conserved phosphatase with broad substrate specificity and diverse cellular functions. We have signed a material transfer agreement with Lixte Biotechnology Holdings, Inc. and obtained the specific PP2A inhibitor (LB-100) which is developed by this company. When we added it to the cell culture medium together with the four transfection factors, it did not exert significant effect on iPSCs formation. However, we found that this drug has some unexpected effect on neural progenitor cells (NPCs). It appears that it can promote the reprogramming of NPCs back to iPSCs.

Santhi Gorantla, PhD (University of Nebraska Medical Center): Humanized Mouse Model to Test Immunogenicity of iPSCs

Goal: Humanized mice as a model to test the immunogenicity of iPS cells. *Aim*: Determine how well the autologous human immune system accepts and supports ex vivo engineered iPSC. Humanized mice with functional immune system will be generated by transplanting CD34+ stem cells isolated from umbilical cord blood into immunodeficient mice. Fibrobalsts isolated from cord tissue will be reprogrammed to iPSC. We will test the formation of teratomas from these iPSC when transplanted into immunodeficient mice with or without a human immune system. Immune responses to the transplanted cells will be assessed by determining leukocyte infiltration, T lymphocyte activation and inflammation.

Results: 1) We successfully generated iPS cells from the fibroblasts by introducing Oct-4, Klf4, SOX-2, and c-Myc (OKSM) genes into the fibroblasts using lentiviral vectors.
2) We performed teratoma assays in humanized mice with autologous iPS in presence of functional human immune system to test their immunogenicity. Our findings suggest that in vitro manipulations to generate iPS cells from fibroblasts did not make them immunogenic, as teratomas were successfully developed from iPS cells in presence of autologous immune system in mice with minimum immune activation. We developed a unique model to validate the prospective utility of iPSC for clinical use. In future we plan to develop disease models with human immune system to test the regeneration capacity of differentiated iPSC for their pre-clinical evaluation.

Woo-Yang Kim, PhD (University of Nebraska Medical Center): Neural Stem Cells and Neurological Disorders

Goals/Aims: Normal brain development requires tight control of neural stem cell self-renewal and differentiation. Abnormal regulation of these processes can lead to structural and functional brain damages and is thereby implicated in neurodevelopmental and neurodegenerative diseases. However, there are difficulties in creating pharmacological interventions for previously known

mechanisms of neural stem cell regulation. The goal of this proposed study is to establish a novel mechanism that can be targeted by currently-available pharmacological drugs. This project particularly aims to 1) Determine the role of the mammalian target of rapamycin (mTOR) in neural stem cell self-renewal in vivo and 2) Establish glycogen synthase kinase-3 (GSK-3) regulation of mTOR signal in neural stem cells.

Results: We generated mTOR mouse models for studying the regulation of neural stem cells in the brain. The deletion of mTOR in the developing neural stem cells led to the formation of smaller brains compared to controls due to the decrease in stem cell proliferation and neurogenesis in the developing brain. We also showed that a key stem cell regulator GSK-3 physically interacted with mTOR upstream components and modulated mTOR activity in neural stem cells. Together, these findings reveal that GSK-3 functionally interacts with a pharmacologically-approachable mTOR signaling and this interaction is important in neural stem cell regulation during brain development.

2011 Stem Cell Subawards

Jung Lim, PhD (University of Nebraska – Lincoln): Controlling Stem Cell Fate via Cell Patterning

Goals/Aims: While mesenchymal stem cells (MSCs) have been recognized as prospective cell sources for cell therapy and regenerative medicine, it is still very challenging to efficiently control MSC fate decision toward the target cell type. To direct MSC function and fate, this study exploited the control of cell-cell interaction via utilizing micropatterning technique. By micropatterning MSCs into interconnected or isolated geometry, we approached to regulate cell-cell interaction, which plays a critical role in cell lineage commitment and differentiation, and examined its effect on MSC lineage commitment to osteogenesis vs. adipogenesis. By evaluating micropatterned cell differentiation and revealing cell signaling mechanism, an integrated picture on how to control MSC behavior was provided.

Results: We aimed to control MSC fate through modulating cell-cell interaction, which plays a critical role in cellular lineage commitment and differentiation. Specific aims included (1) Examine the effect of patterned cell connectivity on hMSC osteogenesis vs. adipogenesis; (2) Determine the role of cadherins in cell patterning-directed hMSC lineage commitment and differentiation. For Aim 1, we achieved establishments of MSC osteogenesis vs. adipogenesis protocols, isolated and intercomnected cell micropatterning, and assessment of cadherin cell-cell adherens protein expression for isolated and connected cells. For Aim 2, we established MSCs with molecularly manipulated cadherins via using small hairpin RNA (shRNA) technique. The co-control of MSC fate decision by geometric cue (micropatterning) and molecular manipulation (shRNA) could be assessed for MSC osteogenesis and adipogenesis.

Mayumi Naramura, PhD (University of Nebraska Medical Center): Regulation of Hematopoietic Stem Cell Homeostatis by CBL

Goals/Aims: 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, successful HSCT requires sufficient number of hematopoietic stem cells (HSCs), a

practical challenge in certain situations. The goal of the research project was to demonstrate that the HSC compartment could be expanded by inhibiting Cbl functions and to lay foundation to exploit this pathway to improve HSCT.

Results: We identified the Cbl family E3 ubiquitin ligases as critical regulators of HSC homeostasis. Studies funded by this grant revealed that Cbl was required to maintain HSCs with the highest regenerative capacity. Additional studies subsequent to this grant period demonstrated that: (1) in the absence of Cbl family proteins, activated tyrosine kinases accumulate (this is consistent with previous studies by us and others); (2) accumulated active tyrosine kinases are thermodynamically unstable and form aggregates inside the cells; and (3) this disrupts proteome homeostasis and adversely affect stem cell functions. Thus, we conclude that Cbl inhibition cannot be used to expand normal HSCs.

Zhao-Yi Wang, PhD (Creighton University School of Medicine): ER-alpha36: Roles in Breast Cancer Stem Cells

Goals/Aims: Specific Aim 1: We aim to investigate the function and the underlying mechanism of ER-α36-mediated non-genomic estrogen signaling in the stem/progenitor cells from ER-positive breast cancer. Specific Aim 2: We aim to investigate the function of ER-α36 in the ER-negative breast cancer stem/progenitor cells and to directly explore the possibility of using ER-α 36 as a target to eradicate breast cancer stem/progenitor cells.

Results: We demonstrated that ER-α36 is the estrogen receptor that mediates mitogenic estrogen signaling in ER-positive breast cancer stem/progenitor cells through coordinated actions of different growth factors and their receptors. Thus, ER-α36 is a critical player in mitogenic estrogen signaling that plays important roles in mammary tumorigenesis and possible in other types of estrogen related tumors as well. We also demonstrated the existence of the ER-α36-EGFR/HER2 positive regulatory loops in ER-negative breast cancer stem/progenitor cells. Disruption of these loops attenuates growth and maintenance of ER-negative breast cancer stem/progenitor cells. These results will greatly advance our understanding of mitogenic estrogen signaling and help to develop novel therapeutic agents for breast cancer.

2010 Stem Cell Subawards

Hesham Basma, PhD (University of Nebraska Medical Center): Induced Pluripotent Stem Cells and COPD

Goals/Aims: Aim#1: Determine the pluripotency and self-renewal capacity of iPS-like cells derived from COPD and non-COPD control lung fibroblasts. Aim #2: Determine if transcription-factor-mediated de-differentiation reprograms functionally abnormal COPD fibroblasts to resemble non-COPD controls.

Results: Aim #1: Two batches were generated from COPD and non-COPD control lung fibroblasts. First batch contains four cell lines while the second batch contains three cell lines from both COPD and non-COPD. The detailed analysis of the first batch revealed incomplete differentiation of this batch due to lacking of a key gene (sox2) expression.

The second batch, however, was successfully reprogrammed to iPS-like cells. Reprogrammed iPSCs were positive for un-differentiation markers *oct3/4, nanog and sox2*, differentiated into cells of the three germ layers and induced teratomas in non-obese diabetic severe combined immune deficient (NOD/SCID) mice. Aim #2: iPS-like cells from the second batch was successfully differentiated into functional fibroblasts that resemble non-COPD fibroblasts more than COPD phenotype. Parent fibroblasts from non-COPD subjects were more active in chemotaxis and gel contraction than fibroblasts from COPD subjects. In contrast, after formation of iPSCs, re-differentiated fibroblasts were similar in function. Analysis of microarrays demonstrated that parent cells of non-COPD were differed by two-fold from COPD cells in expression for 626 genes at p-value < 0.001. In contrast, reprogramming into iPSCs followed by re-differentiation resulted in much more similar gene expression with fibroblasts derived from non-COPD differing by two-fold from those derived from COPD subjects for only 18 genes at p-value < 0.001.

Shi-Jian Ding, PhD (University of Nebraska Medical Center): Proteomic Study of iPS and NPC Cell Induction

Goals/Aims: The long-term goal of this project is to provide stem cell-based reparative therapies for people who suffer from neurotrauma and neurodegenerative diseases. Aim 1. To determine the abundance profiles of proteins in MEFs, MEFs-derived iPS cells and iPS cells-derived NPCs. Aim 2. To determine the abundance profiles of proteins in MEFs, MEFs-derived iPS cells and iPS cells-derived NPCs. Aim 3. To prioritize the candidate proteins targets and conduct orthogonal measurements and initial functional studies with candidate regulatory proteins. 2.1 Prioritize the list of candidate proteins based on bioinformatics analysis and biological interpretation. 2.2 Confirm proteomic results using orthogonal methods (e.g., Western blot, Northern blot, luciferase assay) for interesting candidate proteins in MEFs, MEFs-derived iPS cells and iPS cells-derived NPCs. 2.3 Explore the functional significance of novel candidate proteins by performing genetic manipulation or small molecule inhibition of no more than five candidate proteins.

Results: In this study, 2015 proteins were identified and quantified between MEFs and iPSCs proteome, while 253 and 212 proteins have been identified only in MEFs and iPSCs proteome, respectively. Gene ontology analysis reveals that expressions of many nuclear proteins were enhanced or just started to reprogram MEFs and maintain stemness of iPSCs. The upregulated nuclear proteins in iPSCs include Hdac1/2, Dnmt1, Ccnd1, Smarcc1, and subunits in DNA replication complex. Our proteomic study reveals a biological insight that DNA replication mechanism is enhanced, and Hdac1 plays an important role with promoting Ccnd1 expression and inhibition of Kip family (p21, p27) of cell cycle regulators in the reprogramming process.

John Sharp, PhD (University of Nebraska Medical Center): Mouse and Human iPS Cells: Tools to Probe Cellular Aging

Goals/Aims: The goals of this research project were to determine if bone marrow mono-nuclear cells or hematopoietic stem cells (CD34+HSC) from old human or mouse donors could be re-programmed to induced pluripotent cells (iPSC) and to evaluate whether this re-programming was as efficient for old donor cells, as young donor cells, which had been used previously.

Results: The results of the project, based on in vitro assays, e.g., cell growth, colony formation were that cells from old donors could be successfully reprogrammed to iPSC and this process (using a lentiviral reprogramming kit) was as efficient for old cells as for young cells, despite there being a much higher proportion of senescent (p16 Ink4a+) cells among the old donor cell populations.

2009 Stem Cell Subawards

Iqbal Ahmad, PhD (University of Nebraska Medical Center): Adult Stem Cells for Autologous Cell Therapy

Goals/Aims: Our main goal was to develop safe and reliable approach to generate induced pluripotent stem (iPS) cells from adult sources for autologous stem cell therapy for degenerative blinding diseases such as glaucoma and age-related macular degeneration. We proposed two specific aims to achieve the goal. (1) Conditions for efficient and step-wise conversion of iPS cells into neural progenitors and subsequently into retinal progenitors would be identified. (2) Conditions for efficient and step-wise directed differentiation of LE-derived retinal progenitors into rod photoreceptors and retinal ganglion cells would be identified.

Results: We demonstrated that adult limbal cells could be reproducibly and safely induced into pluripotency by a non-nucleic acid approach. These iPS cells cells are robust source of retinal progenitors from which both photoreceptors and retinal ganglion cells can be generated for autologous stem cell therapy for AMD and glaucoma, respectively.

Andrea Cupp, PhD (University of Nebraska – Lincoln): VEGF Isoform Regulation of Spermatogonial Stem Cells

Goals/Aims: We hypothesized that VEGFA isoforms are important for the early formation and differentiation of spermatogonial stem cells in the testis. The VEGFA gene is composed of different isoforms. VEGFA Isoforms that contain exon 8a are pro-angiogenic and stimulate vascular development. We also propose that they stimulate spermatogonial stem cell differentiation and proliferation. VEGFA isoforms that contain exon 8b are anti-angiogenic and appear to inhibit the actions of angiogenic isoforms. To test this hypothesis we proposed these aims: Aim I. Elucidate the mechanisms of how VEGFA isoforms regulate SSC activity and formation of the SSC population in neonatal mice; and Aim II- Determine the in vivo effects of gonocyte-specific deletion of VEGFA isoforms or the VEGFA co-receptor NRP1 on SSC differentiation, proliferation and testis function.

Results: The results of our research were that VEGFA angiogenic isoforms stimulated spermatogonial stem cell proliferation and antiangiogenic isoforms inhibited sperm stem cells as demonstrated when mice treated with these isoforms had were used in spermatogonial stem cell transplantation experiments. Also we further developed our conditional KO lines of mice and these lines supported this evidence. The pDMRT1 males had alterations in undifferentiated spermatogonial as well as genes that regulated the spermatogonial stem cell niche and reduced fertility. Furthermore knocking out NRP-1 in Sertoli, and Leydig cells had dramatic effects on fertility, undifferentiated spermatogonia and genes that enable the spermatogonial stem cells to

proliferate. Thus, it appeared that we had developed critical mouse models to test VEGFA isoforms effects on spermatogonial stem cell maintenance.

A. Angie Rizzino, PhD (University of Nebraska Medical Center): Human iPS Cell Formation and Sox2-Associated Proteins

Goals/Aims: The goals of this project were to better understand the molecular mechanisms that control the reprogramming of somatic cells into induced pluripotent stem cells and to better understand the molecular mechanisms that control the self-renewal of pluripotent stem cells. To achieve these goals, two aims were pursued: 1) perform unbiased proteomic screens to identify proteins that form high molecular weight protein complexes with the stem cell transcription factor Sox2; and 2) determine whether any Sox2-associated proteins influence the formation and/or the self-renewal of pluripotent stem cells.

Results: 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. In the course of these studies, we identified several proteins required for the self-renewal of pluripotent stem cells. Thus, our study provided new insights into the molecular mechanisms that support the self-renewal of pluripotent stem cells.

Garrett Soukup, PhD (Creighton University School of Medicine): MicroRNA Promotion of Hair Cell Differentiation

Goals/Aims: 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 or multipotent 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.

Results: The project has progressed substantially over the years to include analysis of hair cell-specific factor on different cell types that now include mouse embryonic stem cells (mES cells) and induced multipotent otic progenitor cells (iMOP cells). Work has focused on the effects of a transcription factor (Atoh1) alone or in combination with neurosensory microRNAs (miR-183 family members). Primarily, analysis of gene expression in mES and iMOP cells has indicated that these hair cell-specific factors are more efficacious for inducing expression of hair cell-specific genes when presented in combination, more so in iMOP than mES cells. The results suggest that other factors or processes that transition ES cells toward an otic progenitor fate are required.

Jialin Zheng, MD (University of Nebraska Medical Center): Regenerative Therapy of Parkinson's Disease by iPS Cells

Goals/Aims: The objective is that induced pluripotent stem (iPS) cells will provided a feasible source for mouse embryonic stem (ES)-like stem cells that can be used to create neural stem/progenitor cells (NPCs) and dopaminergic (DA) neurons that can then be transplanted into

the substantia nigra (SN) of Parkinson's disease (PD)-like 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mouse models. 1) We will generate iPS cells and compare their functional properties with mouse ES cells. 2) We will examine the potential of iPS cells to differentiate into NPCs and DA neurons *in vitro* and *in vivo*. 3) We will identify functional, integrated newly-formed neurons developed from intercranially (IC)-injected iPS cell-derived NPCs and DA neurons into the SN and examine the ability of these cells to repopulate the SN and enhance recovery from PD-like symptoms in an MPTP mouse model.

Results: Advances in stem cell research have opened a new era for PD treatment. Specifically, human and murine fibroblast cells have been reprogrammed into induced pluripotent stem cells (iPSCs), which is a cell type suggested for cell therapy in PD. Moreover, we have successfully converted mouse fibroblasts into neural progenitor cell-like cells (Tian et al, 2011 & 2012) with validated neural progenitor cell identify (Tian et al, 2013). We have further improved the reprogramming strategy by directly converting mouse fibroblasts into induced dopaminergic precursors (iDPs), which differentiated into dopaminergic neurons with high efficiency and functionally alleviated the motor deficits, and reduced the loss of striatal DA neuronal axonal termini in a PD animal model (Tian et al, 2015).

Conclusions

The Nebraska Stem Cell Research Project has shown substantial progress and a solid stem cell research foundation has been established. Research has included Parkinson's Disease, auditory hair cell regeneration, chronic obstructive pulmonary disease (COPD), breast cancer, reparative therapies for individuals who suffer from neurotrauma and neurodegenerative diseases, improved strategies for repairing damaged articular cartilage, retina repair, clinical alternatives to creating replacements for restoring normal bone tissues, male fertility and leukemia.

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), National Science Foundation (NSF), Osteology Foundation, Nebraska's Experimental Program to Stimulate Competitive Research (EPSCor) – a National Science Foundation program, and the Bellucci DePaoli Family Foundation.

Progress Report of Funded Subawards

Some of the major highlights of the Nebraska Stem Cell Research Project since 2009:

- Nebraska researchers have received approximately $5 million in additional funds that were directly related to their project. Pending submissions are approximately $10 million.
- Over 75 publications (i.e., articles, manuscripts, papers) have been published or are under consideration for publication.
- Over 40 research positions resulted from these subawards.
- Approximately 80 national and/or international presentations relating to funding from the Nebraska Stem Cell Research Project have been presented.