November 30, 2015

Dear Senator:

In accordance with the Neb.Rev.Stat. §71-8801 et seq, the Stem Cell Research Advisory Committee met May 18, 2015, to determine which of the ten grant applications submitted for 2015 – 2016 should be funded. The following applications were approved for one year with a total amount of funding of $437,000.

Project Title	Principal Investigator	Applicant Organization	Length of Grant	Total Amount
Cancer Stem Cells of Pancreatic Ductal Adenocarcinoma	Dr. A Angie Rizzino	University of Nebraska Medical Center	One Year	$87,400
The Role of RUNXI and GATA2 in Leukemia Stem Cells	Dr. R Katherine Hyde	University of Nebraska Medical Center	One Year	$87,400
Novel Stem Cell Therapy for Parkinson's Disease	Dr. Yuguo Lei	University of Nebraska-Lincoln	One Year	$87,400
Mechanical Stretch Control of Stem Cell Fate	Dr. Jung Lim	University of Nebraska-Lincoln	One Year	$87,400
Mechanisms of VEGFA Isoforms on Germ Stem Cells	Dr. Andrea Cupp	University of Nebraska-Lincoln	One Year	$87,400

These research applications address the following medical areas: (1) pancreatic cancer clinical trials; (2) identifying targets for future drug development in leukemia stem cells; (3) develop stem cell therapy for Parkinson's disease; (4) perspective on how to control human mesenchymal stem cells fate via stretch; and (5) understanding of spermatogonial stem cells (SSC) biology and male fertility. The specific aims and a more detailed explanation of the research studies are attached.

We appreciate the opportunity to administer this grant program and look forward to working with these grantees. If you have any questions, please feel free to contact me.

Sincerely,

Courtney N. Phillips, MPA
Chief Executive Officer
Department of Health & Human Services

Attachments

Stem Cell 2015-01: Dr. A Angie Rizzino, University of Nebraska Medical Center
Cancer Stem Cells of Pancreatic Ductal Adenocarcinoma

Abstract
Many tumors possess a sub-population of tumor cells (Cancer Stem Cells/Tumor Initiating Cells, CSC/TIC) that is solely responsible for continued tumor growth. The gold-standard for proof of CSC/TIC is the Limiting Cell Dilution (LCD) tumor assay, which measures the minimum number of cells need to form a tumor. Importantly, drug-resistance in many cancers appears to be due to the inability of drugs to effectively target CSC/TIC. Surprisingly, the action of drugs used in cancer clinical trials is rarely, if ever, tested against CSC/TIC. The stem cell transcription factor SOX2 has been implicated in the CSC/TIC of ~20 human cancers, including pancreatic ductal adenocarcinoma (PDAC), a highly aggressive malignancy. The vast majority of human PDAC possess mutated and activated KRAS. As a result, these tumors are highly dependent on up-regulated AKT and RAF/MEK/ERK signaling. This has led to multiple PDAC clinical trials testing AKT inhibitors (AKTi) and MEK inhibitors (MEKi). Unfortunately, these drugs have not produced significant responses in PDAC clinical trials, which has led to the general belief that PDAC is largely resistant to AKTi and MEKi. Importantly, elevating SOX2 in many types of cancer cells decreases the efficacy of drugs used clinically. Recently, we determined this is also true for PDAC. Specifically, increasing SOX2 in PDAC cells limits the ability of MEKi and AKTi to inhibit their growth; whereas, knocking down SOX2 enhances growth inhibition of PDAC cells treated with MEKi or AKTi. These studies led to the hypothesis: SOX2 promotes the "stemness" of PDAC and decreases the efficacy of drugs used in PDAC clinical trials by increasing PDAC CSC/TIC.

To test this hypothesis, **three Specific Aims** are proposed. 1) Determine whether altering SOX2 levels alters the number of PDAC cells needed to induce tumors in a LCD tumor assay. 2) Determine whether treatment of PDAC cells with either MEKi or AKTi alters the number of cells needed to form tumors in a LCD tumor assay. 3) Determine whether altering the levels of SOX2 in PDAC cells treated with either MEKi or AKTi alters the number of cells needed to form tumors in a LCD tumor assay. For these studies, a change in the number of cells needed to form a tumor would indicate a change in the percentage of PDAC CSC/TIC. Support for our hypothesis would provide a strong rationale for why MEKi and AKTi have not produced significant results against PDAC in clinical trials and would suggest that targeting SOX2 or its mode of action could improve the efficacy of these drugs. Success in our studies could also radically alter the standard by which drugs used in PDAC and other cancer clinical trials are judged.

Mini-abstract
The proposed studies seek to determine whether the stem cell transcription factor SOX2 reduces the efficacy of drugs used in pancreatic cancer clinical trials by increasing the population of cancer stem cells/tumor-initiating cells in pancreatic tumors.

Stem Cell 2015-02: Dr. R Katherine Hyde, University of Nebraska Medical Center
The Role of RUNX1 and GATA2 in Leukemia Stem Cells

Abstract
Acute myeloid leukemia (AML) is often characterized by the presence of specific, recurrent chromosomal abnormalities. One common subtype of AML is caused by an inversion of chromosome 16 (inv(16)), which generates the fusion gene *CBFB-MYH11*. Current treatments for inv(16) AML include high dose chemotherapy, which is effective in killing the bulk of leukemia cells, but is less successful in eradicating the small population of cells able to propagate the disease, the leukemia stem cell (LSC). Consequently, the majority of inv(16) patients eventually relapse. Therefore, there is great need for new treatments that specifically target the LSCs. Development of such drugs depends on understanding the factors regulating LSC activity.

LSCs share many similarities to normal hematopoietic stems cells (HSCs), including a low rate of proliferation and the ability to self-renew. The transcription factors RUNX1 and GATA2 are known to regulate both these activities in normal HSCs, so may play similar roles in LSCs. Using a mouse model of inv(16) AML, we found that impaired RUNX1 activity results in a decrease in the population of cells enriched for LSCs. In addition, we found that expression of the inv(16) fusion gene induces expression of GATA2, as well as known GATA2 target genes. Based on these findings, we hypothesize that *Runx1* and *Gata2* are required for self-renewal and chemoresistance in inv(16) LSCs. The following specific aims will test this hypothesis

Specific Aim 1: Characterize the role of *Runx1* in inv(16) LSCs. Our previous work implies that *Runx1* is required for the generation of inv(16) LSCs. However, it is not known whether *Runx1* is required for the maintenance of the LSC population. To test this, we will:
1a. Determine the role of *Runx1* in LSC quiescence, self-renewal, and survival.
1b. Test if *Runx1* is required for LSC activity in vivo.
1c. Determine if loss of *Runx1* sensitizes LSCs to chemotherapeutics.

Specific Aim 2: Characterize the role of *Gata2* in inv(16) LSCs. GATA2 is highly expressed in inv(16) leukemia cells from a mouse and humans. Because *Gata2* is required in normal HSCs, we propose that it is similarly required in inv(16) LSCs. To test this, we will:
2a. Determine the role of *Gata2* in LSC quiescence, self-renewal, and survival.
2b. Test if *Gata2* is required for LSC activity in vivo.
2c. Determine if loss of *Gata2* sensitizes LSCs to chemotherapeutics.

Mini-abstract
This project will test the role of two hematopoietic stem cell regulators, RUNX1 and GATA2, in leukemia stem cells with the goal of identifying targets for future drug development.

Stem Cell 2015-04: Dr. Yuguo Lei, University of Nebraska-Lincoln
Novel Stem Cell Therapy for Parkinson's Disease

Abstract:
The proposed project is to develop an efficient therapy for Parkinson's disease using dopaminergic neurons derived from induced human pluripotent stem cells. Recently, we showed that clinical-grade DA neurons could be made from human induced pluripotent stem cells (iPS cells) in large scale, and they were safe and functional to treat PD in animal models. However, only <10% of these cells could survive in vivo due to the anti-survival environment at the transplantation site, preventing translating this approach to PD patients. In this application, we propose to develop a fibrin device with a cocktail of pro-survival factors for simultaneously delivering DA neurons and creating a pro-survival therapeutic niche to enhance the survival of delivered DA neurons for treating PD. In the proposed device, engineered fibrin hydrogel will carry cells and pro-survival factors encapsulated in Poly (lactide-co-glycolide) 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. Three specific aims are proposed.

Aim 1: Engineer a fibrin hydrogel for delivering iPS-derived DA neurons and pro-survival factors. At the therapeutic niche, the engineered fibrin hydrogel will suppress trauma-induced acute inflammation; support the host cell and axon infiltration into the graft, as well as the axon outgrowth from the graft to host tissue; and temporally release pro-survival factors.

Aim 2: Investigate the synergistic effects of various pro-survival factor candidates and find the best pro-survival factor combination for enhancing DA neuron survival in vivo. Two novel in vitro models and the statistical sampling Taguchi Method will be applied to study the synergistic effects and find the best pro-survival factor combo.

Aim 3: Use the engineered fibrin hydrogel to deliver the best pro-survival factor combo and DA neurons to treat PD in rodents. We will test the ability of the pro-survival therapeutic niche for promoting the DA neuron survival as well as the efficacy of the transplanted DA neurons for treating PD in rodents.

Mini-abstract
In this application, we propose to develop a novel stem cell therapy for Parkinson's.

Stem Cell 2015-06: Dr. Jung Yul Lim, University of Nebraska-Lincoln
Mechanical Stretch Control of Stem Cell Fate

Abstract

Tissue engineering approaches have applied mechanical stimulations to human mesenchymal stem cells (hMSCs) and reported improved engineered tissue outcomes. However, there still remains a huge knowledge gap as regards how hMSCs modulate their behavior under mechanical signals. Specifically, while cell stretching has demonstrated its potential to direct hMSC fate, very little is revealed regarding how hMSCs "socially" sense and respond to stretch. The objective of this study is to determine the role of N-cadherin cell-cell adherens junction in stretch-induced hMSC fate decision toward osteogenesis vs. adipogenesis. We test the hypothesis that Ncadherin cell-cell linkage and related mechanosensor, β-catenin, regulate hMSC fate decision under stretch. We will test if cell stretch triggers disassembly of β-catenin from N-cadherin cell-cell junction and induces β-catenin to translocate into nucleus to regulate key transcription factors of hMSC osteogenesis (Runx2) and adipogenesis (PPARγ). For this, hMSCs with molecularly silenced and overexpressed N-cadherin will be exposed to cyclic cell stretching at varying regimens (strain, frequency, etc.).

Following the review of the last year's proposal, we performed key preliminary experiments (cyclic stretch stimulation of hMSC osteogenesis, stretch induction of β-catenin nuclear transfer; see the main proposal) to support the hypothesis and demonstrate the feasibility. We expect based on the preliminary data that hMSCs under mechanical stretch will display increased β-catenin nuclear localization to upregulate Runx2 while suppressing PPARγ, thus triggering hMSC fate into osteogenesis over adipogenesis. The stretch effect is expected to be impaired if N-cadherin cell-cell interaction is silenced. Our study may suggest an innovative role of N-cadherin as a dynamic mechanosensor when stretch directs hMSC fate decision. Results obtained through the analysis of cell stretch, N-cadherin cell-cell junction, and hMSC lineage specification will provide a novel perspective on how to control hMSC fate via stretch.

Also, stimulated hMSC osteogenesis via sensitized N-cadherin stretch mechanosensing may provide advanced protocols for bone tissue engineering.

Mini-abstract

We aim to determine the role of N-cadherin cell-cell adherens junction in mechanical stretch-induced hMSC fate decision into osteogenesis vs. adipogenesis.

Stem Cell 2015-07: Dr. Andrea Cupp, University of Nebraska-Lincoln
Mechanisms of VEGFA Isoforms on Germ Stem Cells

Abstract
Infertility affects 15% of all couples, and half are of male-origin. While research on spermatogonial stem cells (SSCs) has garnered much attention, there is limited understanding of SSC homeostasis to ensure male fertility. However, recent studies from our laboratory have identified a role for vascular endothelial growth factor A (VEGFA) in fertility and, more specifically, in SSC maintenance. Interestingly, the *Vegfa* gene can be spliced into either angiogenic or antiangiogenic isoforms. Cell-specific elimination of either 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.

Consequently, we have developed two aims to further elucidate the mechanisms of how VEGFA isoforms regulate SSC homeostasis.

Aim #1: Determine the direct effects of VEGFA angiogenic isoforms on SSC proliferation. Since Sertoli cells provide the major structural and chemical constituent of the SSC niche, we will harvest germ cells from mice testes with or without Sertoli cell-specific elimination of VEGFA, culture these with or without angiogenic VEGFA164 and will compare their ability to induce cell proliferation pathways as well as colonize recipient seminiferous tubules to determine how the number of SSCs are affected by VEGFA164 rescue.

Aim #2: Determine the potential mechanisms of SSC reduction induced by VEGFA antiangiogenic isoform signaling in male germ cells. Since treatment with VEGFA165b reduced the number of colonies in recipient mice we predict that treatment with VEGFA165b induces apoptosis or autophagy pathways. Thus, we will use germ cells from Sertoli cell-specific knockout of all VEGFA isoforms, and culture them with antiangiogenic VEGFA165b to determine if this treatment directly induces apoptosis in SSCs and if the autophagy pathway is also stimulated. A better understanding of the mechanisms of how VEGFA angiogenic and antiangiogenic isoforms regulate SSC maintenance could allow for therapeutic strategies to be developed in infertile males, as well as potential contraceptive methods and would allow a better understanding of stem cell biology in general.

Mini-abstract
We aim to investigate the mechanisms by which VEGFA angiogenic isoforms promote SSC proliferation as well as how VEGFA antiangiogenic isoforms might be inducing death of SSCs to better understand SSC biology and male fertility.