

ENGAGE

CENter for Stem Cell and Re**Gene**rative Medicine Under**Graduate** Stud**Ent** Summer Program
Funding Application for Summer 2008
Deadline: March 31, 2008

Overview

The Center for Stem Cell and Regenerative Medicine (CSCRM) is a multi-institutional center composed of investigators from Case Western Reserve University, University Hospitals Case Medical Center, the Cleveland Clinic, Athersys, Inc., and The Ohio State University. Building on the 20 year history of adult stem cell research in northeast Ohio, the Center was created in 2003 with a \$19.4 million award from the State of Ohio as a Wright Center of Innovation. An additional \$8 million award in 2006 from the State of Ohio's Biomedical Research and Commercialization Program further validated the Center's ability to achieve its mission to utilize human stem cell and tissue engineering technologies to treat human disease. For more information please visit <http://stemcellcenter.case.edu>.

This is the first year this program is being offered and the purpose of ENGAGE for Summer 2008 is to promote and support undergraduate students' participation in research and creative projects within the field of stem cells and regenerative medicine. Proposed projects will be expected to match the complexity for what is accepted by SOURCE and SPUR funding.

CSCRM brings together established investigators from five institutions with proven expertise in stem cell research and clinical studies. Due to the impressive breadth of investigator expertise, the scope of non-embryonic stem cell types under study within the Center is comprised of nine cell types:

CSC	Cochlear Stem Cells
CTP	Connective Tissue Progenitor Cells
HSC	Hematopoietic Stem Cells
HB1	Hemangioblast (AC133) derived from umbilical cord blood
MAPC	Multi-potent Adult Progenitor Cells
MSC	Mesenchymal Stem Cells
NSC	Neural Stem Sells/oligodendrocyte progenitors
SKMB	Skeletal Myoblasts
UCB	Umbilical Cord Blood Derived Stem Cells

This collection of stem cells is being investigated for therapeutic uses in musculoskeletal, cardiovascular, hematopoietic and neurological disorders.

Orthopedic and Musculoskeletal	CTP, MAPC, MSC, SKMB	Bone and cartilage repair, MSC homing factors, myoblast homing using surface molecule "painting" -- for fractures, joint disease, muscular dystrophy.
Cardiovascular and vascular	HB1, HSC, MAPC, SKMB, UCB	Myocardial regeneration with cell homing, differentiation and regeneration, vascular remodeling -- for ischemic, congestive and dilated cardiomyopathy.

Hematopoietic and cancer	HB1, HSC, MSC, UCB	UCB stem cell expansion and transplantation, cytokine directed differentiation for GVL (graft vs. leukemia), marrow reconstitution and stem cell selection, HSC gene transfer, MSC modulation of GVHD (graft vs. host disease), -- for curing cancer, correction of genetic and immune diseases and protection from chemotherapy.
Neurodegenerative and neurological	MAPC, NSC	Cell implantation into brain and spinal cord for neuronal or glial replacement therapy in Neurodegenerative disorders – Huntington’s disease, multiple sclerosis, amyotrophic lateral sclerosis and spinal cord injury.

Applicants should review the CSCRM directory below to determine potential projects and mentors. **Applicants will need to choose a therapeutic area in Biology and Immunology, Cardiovascular, Dermatology, Imaging, Musculoskeletal, Neurological, Oncology, Reproductive or Sensory** and the ENGAGE Administrator, Michael Gilkey, will assign them to a principal investigator in that area. If there is an investigator the applicant especially desires to work with please mention them on the form. As applicants are assembling their project description and objectives, please feel free to use <http://ora.ra.cwru.edu/stemcellcenter/howeare/howeare.htm> to investigate the CSCRM membership and contact our investigators directly. Abstracts of previously funded projects by SOURCE or SPUR with participation by some of our members are listed at the end of this document.

Eligibility

All undergraduate students at Case Western Reserve University who are continuing as undergraduates at CWRU in Fall 2008 are eligible to apply for ENGAGE summer funding. Award recipients must be registered for fall classes prior to beginning the summer project. Projects must be completed at CWRU, Cleveland Clinic, University Hospitals Case Medical Center or Athersys. To this extent, students may apply for funding to support research and creative projects, including stipend and materials. Students who are awarded full research stipends (\$3,500) cannot enroll in more than one summer class. While students may apply for funding for different projects, students may not accept funding for 2 different projects (that is, for example, students may not be part of the SPUR program and receive ENGAGE funding for a separate project). If you are accepted into more than one project program, you must choose in which project you want to participate. Material costs of up to \$1500 will be covered by ENGAGE and funds will go directly to the mentor assigned to the student.

Award Description

The CSCRM office recognizes and is grateful to the following for Summer 2008 funding:

- *CWRU Provost Funding* – All undergraduate students are eligible for awards. Ten awards of up to \$5000 will be given.

Application Procedure

A completed application packet consists of the following:

- 1) The ENGAGE summer funding application
- 2) Official Academic Transcript
- 3) A brief (approximately 2-3 pages) proposal that addresses the following:
 - Project Title
 - Goals and Objectives
 - Project Description (please review the abstracts at the end of this document)
 - Discussion of estimated time commitment required for the project
 - Discussion of how this project is part of the student’s overall educational plan and goals
- 4) Resume

Also, see the SOURCE website for SOURCE Summer Funding Hints (<http://www.case.edu/provost/source/opp/funding.htm>)

Deadline

The deadline for submitting a completed Application Packet, which includes application form, transcript, proposal and resume is **March 31, 2008**.

Selection Criteria

Applications will be reviewed to confirm that the applicant meets the eligibility criteria. The selection committee will evaluate application files and identify recipients based on the strength and educational value of the proposal, the do-ability of the project, and ability to match with an appropriate mentor.

Please send completed form to Michael.gilkey@case.edu or campus mail to Michael Gilkey, Wolstein Research Building 2-501, no later than March 31, 2008.

Questions regarding this program can be directed to Michael Gilkey via email or phone, 216-368-2079.

The Center for Stem Cell and Regenerative Medicine Directory includes:

Investigator	Expertise	Investigator	Expertise
Biological and Immunology			
K. Asosingh, CC	Stem cell biology in lung diseases	L. Lasky, OSU	UCB expansion and bioreactor design
R. Beck, Case	Immunology of bone marrow transplantation	J. Maciejewski, CC	Stem cell failure syndromes
K. Bunting, UHCMC	Stem cell signaling in HSCs	E. Medof, Case	Pathology, Stem cell biology
A. Hijaz, UHCMC	Homing of MSCs for urinary incontinence	C. O'Keefe, CC	Genomic damage in HSC
J. Jacobberger, Case	Flow imaging of stem cell survival and differentiation	S. Qian, CC	Development of stem cells for islet transplantation
Cardiovascular			
A. Askari, CC	Myocardial vascular repair	M. Penn, CC	Myocardial regeneration and repair
R. Deans, Athersys	MAPCs for myocardial regeneration	R. Perry, Athersys	MAPCs for myocardial regeneration
P. DiCorleto, CC	Endothelial cell function, vascular cell signaling/gene expression	V. Pompili, OSU	Myocardial vascular repair
S. Ellis, CC	Stenting, therapeutic angiogenesis and coronary revascularization	D. Simon, Case, UHCMC	Developing new approaches to targeting restenosis of arteries
E. Elgudin, Case, UHCMC	Circulatory support, biological tissue substitutes and tissue engineering	N. Smedira, CC	Heart transplantation and ventricular assist devices
M. Jain, Case, UHCMC	Regulating factors involved in antithrombotic endothelial surfaces	A. Ting, Athersys	MAPCs for myocardial regeneration
K. Laurita, Case, MetroHealth,	Imaging and mechanisms of cardiac impulse	W. Van't Hof, Athersys	MAPCs for myocardial regeneration
J. Navia, CC	Heart disease and surgical techniques	H. von Recum, Case	Tissue engineering blood vessels
P. Parsons, NASA	Measurement of blood vessel growth and remodeling		
Dermatological			
R. Atit, Case	Dermal precursor from MSC	A. Gilliam, Case	Dermatology, lymphoma and autoimmune diseases
K. Cooper, Case, UHCMC	Skin remodeling with progenitors		
Imaging			
J. Duerk, UHCMC	Imaging core for all in vivo modeling, especially HSC, MSC	Z. Lee, UHCMC	Imaging core for all in vivo modeling, especially HSC, MSC
Musculoskeletal			
E. Alsberg, Case	Cell-instructive biomaterials, drug delivery, musculoskeletal engineering	C. McDevitt, CC	Cell-matrix interactions in the repair of connective tissues
A. Caplan, Case	Discovered MSC, founded Osiris	R. Midura, CC	Bone repair
J. Dennis, Case	MSC culture, differentiation and histology	G. Muschler, CC	Marrow connective tissue progenitor isolation
E. Greenfield, UHCMC	Bone regeneration from progenitors	L. Solchaga, Case	Cell-based tissue engineering with MSC
N. Harter, Case	Muscle cell pathways for cell cycle restoration	M. Zborowski, CC	Separation processes for medical applications
S. Haynesworth, Case	MSC characterization and stromal support, co-founder Osiris	M. Tate, Case	Cell-based tissue engineering for bone repair
T. Hering, Case	Cartilage chondrocyte biology		
Neurological			
M. Damaser, CC	Biomechanics and neuromuscular control of the lower urinary tract and female pelvic floor	M. Siemionow, CC	Microsurgery, hand surgery, peripheral nerve surgery
D. Janigro, CC	Stroke and cerebrovascular damage	J. Silver, Case	Spinal cord injury and repair
W. Macklin, CC	Neurodegenerative diseases	B. Trapp, CC	Neurodegenerative diseases, multiple sclerosis
R. Mays, Athersys	MAPCs used to treat neurological diseases	G. Wnek, Case	Use of nanofiber scaffolds to restore function in CNS-related diseases
R. Miller, Case	Spinal cord diseases and oligodendrocyte repair		
Oncology			
J. Auletta, Case, UHCMC	Antigen presenting cells and MSC in immunomodulation	M. Parat, CC	Endothelial cell migration and angiogenesis in breast cancer
S. Gerson, Case, UHCMC	Stem cell gene transfer, drug selection, transdifferentiation	C. Qu, Case	Signaling mechanisms of hematopoietic cell processes
J. Harrington, Athersys	Technology development, novel drug target identification	N. Sizemore, CC	Gene expression, oncogenesis, and apoptosis in cancer
Y. Kwon, Case	Drug delivery and cancer immunotherapy and bioreactor design	N. Sreenath, Case	Signal transduction in cancer and inflammation
M. Laughlin, Case, UHCMC	UCB for hematopoietic and vascular regeneration, transplantation	W. Tse, Case, UHCMC	Lymphoma, leukemia, and multiple myeloma
H. Lazarus, UHCMC	Clinical MSC and HSC transplantation	M. Veigl, Case	Mutagenesis by chemotherapeutic agents
M. Mersarovic, Case	Systems biology and mathematical modeling	Y. Yang, Case	Cytokine signal transduction
Reproduction			
J. Liu, Case, UHCMC	Reproductive endocrinology and infertility and in vitro fertilization	N. Rote, Case, UHCMC, MetroHealth	Reproductive immunology and human trophoblast differentiation
K. Molyneaux, Case	Development of the mammalian gonad		
Sensory			
K. Alagramam, Case, UHCMC	Genes to develop, protect and regenerate sensory hair cells	N. Waheed, CC	HSCs to treat retinopathy
B. Anand-Apte, CC	Ocular angiogenesis		

Case (Case Western Reserve University), CC (the Cleveland Clinic), UHCMC (University Hospitals Case Medical Center), and OSU (Ohio State University)

Projects Funded by SOURCE or SPUR

#20. The ABCs of M-S-Cs and P-E-R-I-C-Y-T-E-S

Canting Guo, Donald P. Lennon, Marilyn A. Baber, J. Michael Sorrell, and **Arnold I. Caplan**.

Mesenchymal Stem cells (MSCs) are multipotent progenitor cells that express a multitude of phenotypes, including osteoblasts (bone forming cells), chondrocytes (cartilaginous matrix forming cells), myoblasts (muscle forming cells), adipocytes (fat forming cells), tenocytes (tendon forming cells), and fibroblasts. They are hypothesized to reside in various tissues such as fat, bone marrow, skin, vasculature, and muscle in order to be available to aid in tissue repair, regeneration and turnover. When MSCs differentiate down their multiple lineages they are associated with the recruitment of blood vessels, which lends support to the idea that mesenchymal stem cells are pericytes, vascular support cells that endothelial cells summon to cover the blood vessels. Previous research indicate that pericytes have osteogenic capability (Doherty et al. 1998, Brighton et al. 1992) and preliminary studies suggest that bone marrow-derived MSCs have the potential to be pericytes. The Caplan lab has studied only bone marrow-derived mesenchymal stem cells (BMMSCs) and these serve as the standard to determine if MSCs isolated from other tissues have the capacity to serve as pericytes. This study investigates whether adipose derived mesenchymal stem cells (AMSCs) can exhibit such pericyte potential. To test the hypothesis, a side-by-side comparison of adipose and bone marrow derived mesenchymal stem cells was conducted. The bone marrow and adipose derived mesenchymal stem cells were each cultured in four distinct assays: osteogenic, chondrogenic, adipogenic, and vascular. The first three assays induced the MSCs to differentiate toward their respective end products. The vascular assay tested the MSCs' ability to align with endothelial cells, while the endothelial cells formed structures resembling the walls of blood vessels. From these assays we discovered that the AMSCs differentiated into osteoblasts, adipocytes, chondrocytes, and lined up along the endothelial cells. However, the AMSCs did not show the same differentiation pattern that the BMMSCs did. For example, the osteogenic AMSCs exhibited a decreased level of alkaline phosphatase activity and the adipogenic AMSCs did not form as many fat droplets as the BMMSCs did. Lastly, more BMMSCs arranged themselves along the endothelial cells than the AMSCs did. Much of the disparity can be attributed to the fact that the AMSCs were subjected to conditions optimized for bone marrow derived mesenchymal stem cells. Despite the malnutrition, the AMSCs managed to align with the endothelial cells, supporting the hypothesis.

#30 Lateral plate mesoderm fate mapping and possible lineage derivatives in the mouse embryo.

Hilary Michel, Ozimba Anyangwel, **Radhika**

The dermis is one of the two main layers of skin that lies directly below the outer-most epidermis. It is a vital component of the skin as it allows for wound healing, skin patterning (i.e. why the skin on your belly is different from the skin on your face), and the development of hair follicles, sweat and oil glands. In addition, it serves as a barrier to the outside world and houses the nerves and blood vessels of the skin. Knowing of the skin's vast importance within an organism, it is pertinent that we discover some currently unknown information: the origins of the dermis and other dermal components in mammals. In the chick, ventral/belly side dermis originates from the lateral plate mesoderm (LPM), a transitory structure that exists on the lateral edges of the embryo and then differentiates into other tissues. In our study in the mouse embryo, we hypothesized that LPM also gives rise to ventral dermis and other components of the skin in mammals. We found genetically LPM-lineage labeled cells give rise to ventral dermis and to several new tissues not previously known, like dorsal dermis, brown fat, and skeletal muscle. In order to define the identity of these lineage-labeled cells, we conducted marker analysis with fluorescent antibodies. Our results showed that the vast majority of lineage-labeled cells in the brown fat and dorsal dermis were double-labeled as endothelial cells as well. However, a large number of lineage-labeled cells in the ventral skeletal muscle were not endothelial cells. Ongoing experiments will determine if the lineage-labeled cells in the muscle are satellite cells or actual muscle fibers. Knowing where the dermis comes from and understanding the path the dermal

cells undergo to get to their final destinations will give us clues to the signaling pathways that are relevant to cell differentiation, identity, and fate.

#33 P140K MGMT Gene Transfer Model to Protect Hematopoietic Stem Cells from Alkylating Chemotherapeutic Damage

Myles S. Nickolich, Jane Reese Koc, Colin L. Sweeney, and **Stanton L Gerson**

Preventing chemotherapy's harmful side effects on healthy cells has been better realized with the advancement of gene therapy. Temozolomide (TMZ) is a chemotherapy drug that disrupts normal cell function by alkylating guanine at the O6 position. DNA alkyltransferase (AGT), the gene product of O6-Methylguanine DNA methyltransferase (MGMT), removes alkylations at the O6 position on guanine. Gene transfer of a mutant form of MGMT (PI40K MGMT) confers resistance to the AGT inhibitor O6-benzylguanine (BG) and TMZ. CD34+ hematopoietic stem cells transduced with integrating vectors such as gamma-retrovirus and lentivirus containing MGMT has shown promising results in protecting bone marrow from the toxic effect of alkylating agents, resulting in enrichment of transduced cells and potentially enhancing the effectiveness of chemotherapy against tumors. We are proposing a clinical trial using lentiviral gene transfer of MGMTPI40K into autologous CD34 cells. However, the use of vectors that integrate into the genome poses a safety concern. Recently, a gene transfer clinical trial conducted in France on children showed that gamma-retroviral insertions caused an increase in oncogenicity following treatment. Lentiviruses, while similar to gamma-retroviruses in infection method, are thought to decrease the potential for oncogenicity by inserting inside gene coding regions, rather than upstream of coding regions as do retroviruses. We sought to address safety concerns by limiting the number of insertions per cell and characterizing the location of insertions. We tested a range of virus doses to determine which multiplicity of infection (MOI) resulted in a high degree of drug resistance with less than 2 insertions per cell. We assessed drug resistance by transduction rate and in vitro drug resistance. Insertion site analysis was determined using linear amplification mediated polymerase chain reaction (LAM-PCR). We found an MOI of 75 to be optimal with a 42% +/- 3 gene transfer rate and an average of 1.5 +/- 5 insertions per cell in three patients. The percentage of cells harboring PI40K MGMT increased to 100% after treatment with BG and TMZ, indicating selection for transduced cells. Based on these data, we have shown that enrichment of cells with MGMT PI40K is attainable and are recommending an MOI of 75 for clinical studies. We are currently sequencing approximately 100 insertion sites to determine whether they are in "safe" genomic regions rather than "unsafe" promoter regions in accordance with FDA recommendations.

Role of IHMG-CoA Reductase In Mouse Germ Cell Migration

Jiad Ding, Jennifer Nalepka, Brian Dudley, **Dr. Kathleen Molyneaux**.

During embryonic development, primordial germ cells (PGC), the precursors to eggs or sperm, multiply and move through the hindgut towards the genital ridge. Defects in PGC migration and proliferation may result in infertility or germ cell tumors. Past research has demonstrated that hydroxymethylglutarylcoenzyme A reductase (HMG-CoA reductase), a regulatory enzyme in cholesterol and isoprenoid biosynthesis, is necessary for correct germ cell migration in fruit flies and zebrafish. To investigate whether this enzyme contributes to PGC development in mammals, the distribution of HMG-CoA reductase was first examined in mouse embryos. Quantitative RT-PCR revealed that HMG-CoA reductase mRNA is uniformly expressed in E9.5 mouse embryos with no significant increase in the target genital ridges when compared to neural and gut tissue. This resembles HMG-CoA reductase expression in zebrafish, but is unlike that in flies where the enzyme is elevated in the somatic gonadal precursors. Upon staining embryo slices with filipin, no significant difference in total cholesterol was identified throughout the tissue, thus indicating that HMG-CoA reductase activity is also uniform. However, a cholesterol sensitive bio-probe detected heightened levels of membrane cholesterol in the genital ridges. Together, these results suggest that gonadal development may require cholesterol trafficking in the ridge. Additionally, we inhibited HMG-CoA reductase functionality with statins to further test the role of the enzyme in PGC migration. In cultured mouse tissue, stain treatment

reduced PGC numbers in a dosage-dependent manner and induced widespread apoptosis. Further studies will seek to address whether HMG-CoA reductase modulates germ cell survival through the cholesterol or isoprenoid pathway.

Fluid Volume Conductance for Determination of Bladder Volume

Bradley C. Gill, Paul C. Fletter, Paul J. Zaszczurynski, Alfred Perlio, Daniel Yachia, **Margot S. Damaser**

Clinical urodynamics is the present standard for diagnosing bladder voiding dysfunction. The nonphysiological nature of this exam often hinder symptom reproduction in the clinical setting. Currently, a small device is being developed to conduct ambulatory urodynamics from within the bladder. This study investigates the feasibility of using fluid volume conductance for the real-time intravesical volume measurement needed in urodynamics. Devices tested consist of polymer bodies having 4 electrodes. Electrode configurations and probe geometries were tested in bladder-like latex vessels using saline having conductivity similar to urine. Sensitivity to temperature and fluid concentration were determined using Tyrodes solution and fresh pig bladders in vitro. The voltage across the fluid volume was found to be inversely related to volume. The ideal probe configuration was found to be an ellipsoid having strip electrodes spaced at 25 degrees. Increasing fluid temperature and concentration increased solution conductivity, decreasing the measured voltage. Urine's dynamic chemical properties therefore necessitate real-time compensation of conductivity for clinical application which could be accomplished with another smaller electrode array.

Wnt Signal Transduction in Craniofacial Dermal Development

Preethi Mani, John Myers, **Radhika Atit**

The dermis houses the hair follicles, glands, and blood vessels of the skin and is critical to the functioning of skin. In this study we focus on the development of craniofacial dermis. In the chick embryo, craniofacial dermis is derived from cranial neural crest cells (CNC). The signals that instruct a CNC to become a dermal cell, however, are unknown. The Wnt signaling pathway specifies dermal fate in the dorsum of the mouse. Although dorsal dermis is derived from the somites, we hypothesize that there is a conserved mechanism of Wnt signaling in the development of craniofacial dermis from CNC. To test this hypothesis, we first characterized when and where Wnt signaling occurs in the head during murine development. We found that Wnt signal is especially strong in the C and dermal progenitors, as well as in the subectodermal cells of the branchial arches, the ear, and the brain. Beta-catenin is a central transducer of Wnt signaling; the absence of beta-catenin in cranial neural crest cells leads to a loss of Wnt signaling activity and loss of craniofacial dermis. Our data demonstrate a strong role for Wnt signaling in craniofacial dermal development. In the future, we will continue to examine the mechanism Wnt signaling in craniofacial dermal development. By defining the signals of dermal induction in different parts of the embryo, we can create functional skin equivalents to treat congenital skin defects, severe burns, and wounds.

