THE FIRST MIDWEST CONFERENCE ON STEM CELL BIOLOGY AND THERAPY

MEADOW BROOK HALL

OAKLAND UNIVERSITY, ROCHESTER, MI
May 9–11, 2008

CONFERENCE PROGRAM

JOINTLY SPONSORED BY:

Oakland University

Beaumont Hospitals

IN COOPERATION WITH:

St John Providence

www.oakland.edu/research/stemcell

CONFERENCE SUPPORTERS

PLATINUM

Sigma Life Science

Oakland University's School of Health Sciences

Oakland University's College of Arts and Sciences

GOLD

Oakland University's School of Nursing

College of Arts and Sciences
School of Health Sciences
School of Nursing

SILVER

Blackstone Medical, Inc.

Biospherix

BRONZE

Center for Organogenesis, University of Michigan

American Type Culture Collection (ATCC)

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Please Turn-In Exit Survey at Registration Desk

WELCOME

THE FIRST MIDWEST CONFERENCE ON STEM CELL BIOLOGY AND THERAPY

Statement:

The SCBT Conference provides an opportunity to bring together scientists and clinicians from across the globe to share the latest advancements in stem cell research.

Conference Topics Include:

- Basic stem cell biology
- Stem cell therapeutics
- Stem cell-based tissue engineering
- Drug discovery and design
- Ethical and political issues

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ORGANIZATION COMMITTEE

G. Rasul Chaudhry

Chair

Sumi Dinda — **Coordinator**

Jaime Brozowski — **Secretary**

Members

Ibrahim Ibrahim, Sue O'Shea, Ivan Maillard, Omar A. Khan, Shravan Chintala, Mick Perez-Cruet, David Felten, Anne Mitchell, Kenneth Mitton, Mike Poosch, Dave Svinarich, Mike Trese, Dong Yao

LETTER FROM THE GOVERNOR

JENNIFER M. GRANHOLM
GOVERNOR

STATE OF MICHIGAN
OFFICE OF THE GOVERNOR
LANSING

JOHN D. CHERRY, JR.
LT. GOVERNOR

May 9, 2008

Dear Friends:

It is my privilege to welcome you to the First Midwest Conference on Stem Cell Biology and Therapy. This event offers an opportunity to discuss developments in stem cell research with scientists and leaders in the field, and I am certain that the presentations and education sessions will be beneficial for all.

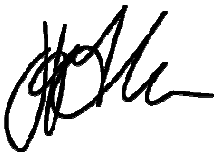
I wish to commend the conference, its participants and hosts for encouraging research, disseminating information, and promoting education relating to stem cells.

This year, Senator Gretchen Whitmer and Representative Andy Meisner introduced bills to remove restrictions on embryonic stem cell research. Michigan's limitations on stem cell research are stricter than federal policy and it is my hope to change this. We need to tap the full power of this modern science to combat life-threatening illnesses. Michigan has great universities and world renowned scientists with the knowledge and ability to make embryonic stem cell cures a reality.

The exciting potential that stem cell research holds for curing many diseases and disorders is a tremendous source of enthusiasm and hope. Thank you all so much for your commitment and dedication to good health. May this be the first of many Midwest Conferences on Stem Cell Biology and Therapy.

Again, welcome and please accept my very best wishes for an informative and educational event.

Sincerely yours,



Jennifer M. Granholm
Governor

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LETTER FROM THE CHAIR

May 9, 2008

A Personal Message from the SCBT Conference Chair

It is with great pleasure that I extend my sincerest greetings to those attending the First Midwest Conference on Stem Cell Biology and Therapy (SCBT) hosted by Oakland University and Beaumont Hospitals, in cooperation with St. John Providence Hospital.

This conference presents an ample opportunity to converse with stem cell researchers, experts, and leaders, who have lead the innovations in biomedical and life sciences, and serves as an excellent source of motivation for students and investigators alike. Oakland University is the second fastest growing state university in Michigan, which prides itself on encouraging not only graduate students, but also undergraduate students, to participate in high-level, creative research projects, gaining hands-on experience. Therefore, the participation of students and junior researchers is an integral part of the SCBT conference.

I wish to commend the SCBT conference organization committee, speakers and attendees for adding to the success of this splendid event.

I hope you may enjoy the sights of Oakland University's historic campus in Rochester. Please accept my sincere best regards for this educational conference.

Sincerely,

G. Rasul Chaudhry, Ph.D.
Chair of SCBT Conference
Professor of Molecular Biology & Stem Cell Research
Oakland University

CAMPUS MAP

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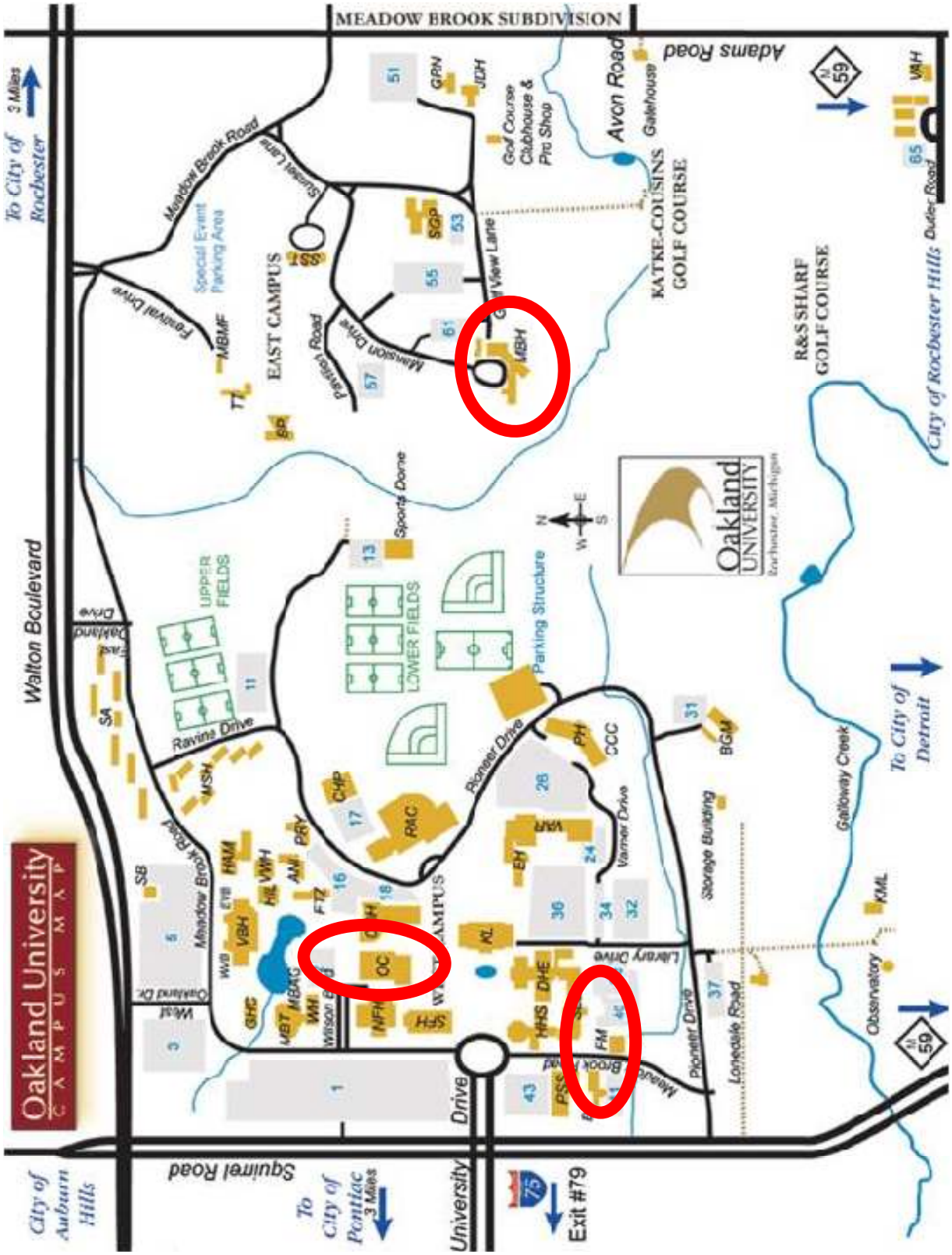
CONFERENCE MAP

CAMPUS MAP

Circled Areas Indicate Conference Areas: SEB- Science and Engineering Building; MBH- Meadow Brook Hall; OC- Oakland Center



CONFERENCE MAP

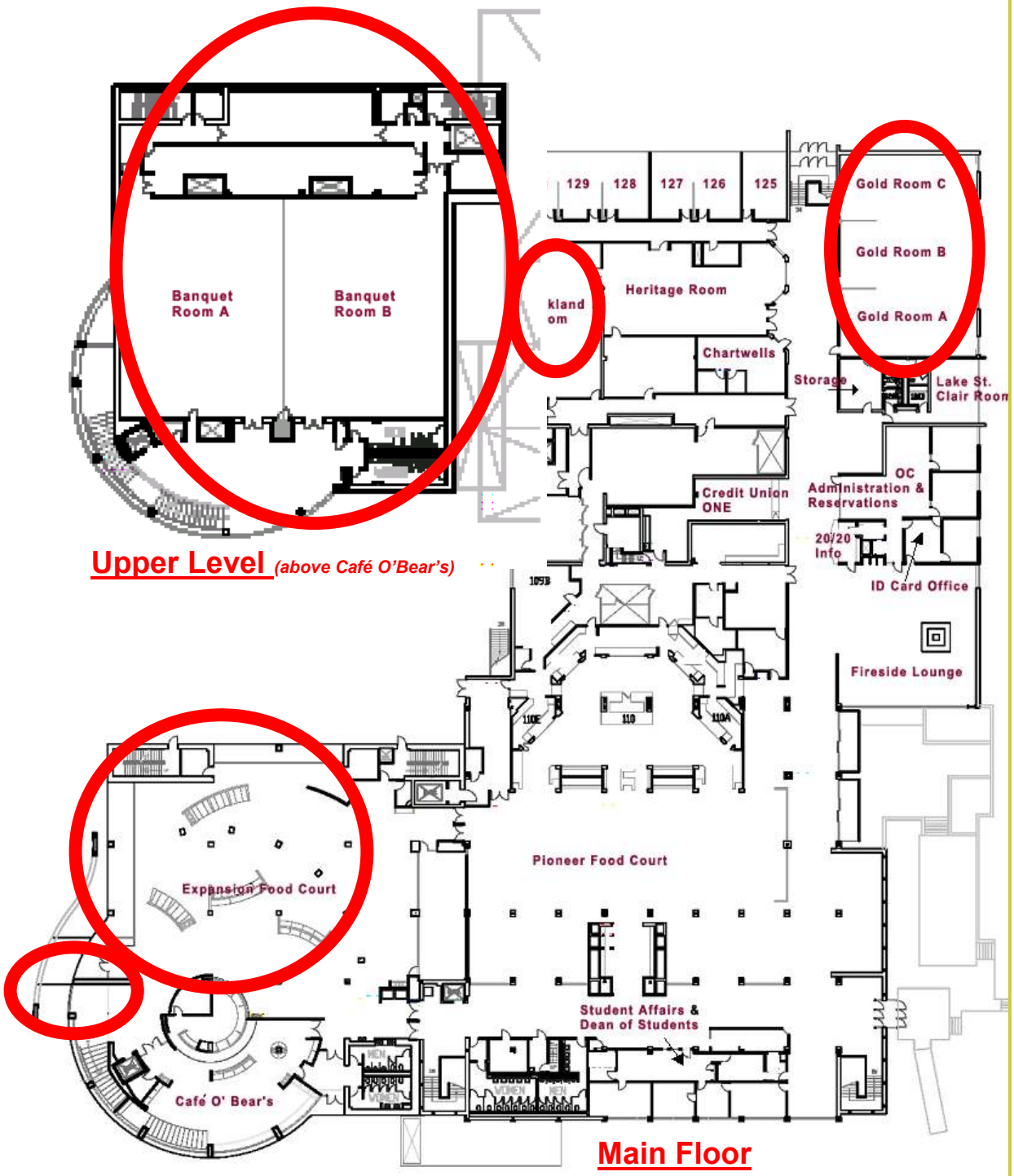
Circled Areas Indicate Conference Areas

Upper Level
Upper level features:

- Banquet Rooms A and B

Main Level
Main level features:

- Café O' Bear's
- Expansion Food Court – Poster Exhibit



SPEAKER PRESENTATION LIST

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Presentations will be given by:
(speakers in alphabetical order)

Kameswara Badri, Ph.D., Wayne State University
Thomas Bauer, Ph.D., NCI, NIH, DHHS
Jaime Brozowski, B.S., Oakland University
Asamoah Bosomtwi, M.S., Oakland University

Michael Chancellor, M.D., Beaumont Hospitals
C.C. Chang, Ph.D., Michigan State University
Chong Chen, B.S., University of Michigan
Michael Chopp, Ph.D., Henry Ford Hospital/OU
Mick Perez-Cruet, M.D., M.S. Providence Hospital

Luis Villa Diaz, Ph.D., University of Michigan
Thomas Diekwisch, D.M.D., Ph.D., University of Illinois at Chicago College of Dentistry
Kimberly Drenser, M.D., Beaumont Hospitals
Gregory Dressler, Ph.D., University of Michigan

William Fairbrother, Ph.D., Brown University
Christopher Fecek, B.S., Oakland University
Shannon McKinney-Freeman, Ph.D., Harvard Medical School & Children's Hospital
Mark Furth, Ph.D., Wake Forest Institute for Regenerative Medicine

Christine Gorka, Ph.D., Providence Hospital
Theresa Gratsch, Ph.D., University of Michigan
Deborah Gumucio, Ph.D., University of Michigan
Vilma Yuzbasiyan-Gurkan, Ph.D., Michigan State University

Gary Hammer, M.D., Ph.D., University of Michigan
Ales Hampl, D.V.M , Ph.D., Institute of Experimental Medicine & Masaryk University, Czech Republic

Ibrahim Ibrahim, M.D., M.P.H, Ph.D., Beaumont Hospitals

Omar Khan, M.D., Wayne State University Medical School

Senator Carl Levin, D-Michigan
Tenneille Ludwig, Ph.D., University of Madison-Wisconsin
Domenico Luongo, M.S., **C.H.M.M.**, Oakland University

Ivan Maillard, M.D., Ph.D., University of Michigan
Representative Andy Meisner, D-Ferndale, Michigan
Jack Mosher, Ph.D., University of Michigan

Andras Nagy, PhD., Mount Sinai Hospital, Toronto

Jack Parent, M.D., University of Michigan
Graham Parker, Ph.D., Children's Hospital of Michigan
Bruno Peault, Ph.D., University of Pittsburgh & Children's Hospital of Pittsburgh
José Garcia Perez, Ph.D., University of Michigan

Pamela G. Robey, Ph.D., NIDCR, NIH

Peter Sartipy, Ph.D., Cellartis AB, Sweden
Jill Slater, B.S, Wayne State University
David Svinarich, Ph.D., Providence Hospital

Shuichi Takayama, Ph.D., University of Michigan
Dean Tantin, Ph.D., University of Utah
Barbara Tigges, Ph.D., BioE, Inc. Research and Development

Donggang Yao, Ph.D., Georgia Institute of Technology
Sichang Zhou, B.S., Wayne State University

CONFERENCE SCHEDULE

CONFERENCE SCHEDULE

FRIDAY, MAY 9

Location: Oakland Conference Center- Main Floor
10:00 a.m. – 4:00 p.m. On-Site Registration

Location: Oakland Conference Center- Expansion Food Court
2:00 – 4:00 p.m. Poster and Exhibit Set-up

4:00 – 5:00 p.m. Poster and Exhibit Viewing

Location: Meadow Brook Hall
6:00– 8:00 p.m. **Opening Session**

Welcome *by G. Rasul Chaudhry Ph.D., Oakland University and Ananias Diokno , M.D., Chief Medical Officer, Beaumont Hospital*
Introduction *by Virinder K. Moudgil, Ph.D., Senior Vice President of Academic Affairs and Provost, Oakland University*

- **Keynote Speaker, Honorable Senator Carl Levin**, D-Michigan, "The gift of life: Pursuing ESC research in Michigan"
- **Ales Hampl, D.V.M , Ph.D.**, Institute of ExperimentalMedicine and Masaryk University, Czech Republic, "How do hESC sense and respond to damage to their DNA?"
- **Michael Chopp, Ph.D.**, Henry Ford Hospital & Oakland University, "Remodeling injured brain with cell therapy"

8:30 – 10:00 p.m. Poster Awards and Reception

9:00 – 9:45 p.m. Jr. Investig. & Students Meet the Experts

SATURDAY, MAY 10

Location: Oakland Conference Center- Banquet Room(s)
7:00 – 10:00 a.m. On-Site Registration

7:00 – 7:30 a.m. Poster and Exhibit Set-up

7:00 – 8:00 a.m. Continental Breakfast

7:00 – 8:00 a.m. Jr. Investig. & Students Meet the Experts

8:00 – 8:15 a.m. **Introduction and Welcome (Banq A)** *by Kathleen Moore, Ph.D.,Associate Dean, College of Art & Sciences, Oakland University*

8:15 – 10:00 a.m. **Stem Cell Biology – Session I (Banq A)**
Moderator: *Dave Svinarich, Ph.D., Director of Patient Care Research, Providence Hospital*

- **Tenneille Ludwig, Ph.D.**, University of Madison-Wisconsin, "Feeder-free culture of embryonic stem cells"
- **Gregory Dressler, Ph.D.**, University of Michigan, "Renal epithelial stem cells and development"
- **Kameswara Badri, Ph.D.**, Wayne State University, "From bronchial myogenesis to the TIP family of transcription regulators"
- **Ivan Maillard, M.D., Ph.D.**, University of Michigan, "To be or notch to be: What notch does and does not do in the hematopoietic system"

FRIDAY, MAY 9

Location: 372 Science & Engineering Building; 3rd Fl

Workshop ***(Registration- Oakland Conference Center)***

11:30 –12:15 p.m. Lunch

12:30 – 1:30 p.m. Regulatory

- **David Svinarich, Ph.D.**, Providence Hospital
- **Domenico Luongo, MSc**, **CHMM**, Oakland University

1:40 – 2:40 p.m. Basic Science
Stem cell isolation, culture, maintenance and differentiation

- **Jaime Brozowski, BS**, Oakland University
- **Christopher Fecek, BS**, Oakland University
- **Theresa Gratsch, Ph.D.**, University of Michigan

2:40 – 3:00 p.m. Coffee Break

3:00 – 4:00 p.m. Clinical
Animal and human studies

- **Michael Chancellor, MD**, Beaumont Hospitals, "Muscle stem cell tissue engineering for urinary incontinence"
- **Ibrahim Ibrahim, MD, MPH, Ph.D.**, Beaumont Hospitals, "Funding for stem cell therapy research"

8:15 –10:00 a.m. **Stem Cell Therapy – Session I (Banq B)**
Moderator: *Mick Perez-Cruet, M.D.,M.S., Neurosurgery, Providence Hospital & Oakland University*

Degenerative/ Age-related Diseases

- **Omar Khan, MD**, Wayne State University Medical School, "The use of SC therapy in multiple sclerosis: Promises and challenges"
- **Jack Parent, MD**, University of Michigan , "hESC-derived neural progenitor isolation for regenerative therapy after stroke"
- **Mick Perez-Cruet, MD**, Providence Hospital, "Stem cell use in degenerative disc disease"
- **Kimberly Drenser, MD**, Beaumont Hospitals, "Retinal Development"

SATURDAY, MAY 10 CONTINUED

10:00 – 10:20 a.m. Coffee Break

10:00 a.m – 12:00 p.m. Poster and Exhibit Viewing

10:20 – 11:50 a.m. **Stem Cell Biology – Session II (Banq A)**
Moderator: *Deborah Gumucio, Ph.D., Director of Center for Organogenesis, University of Michigan Medical School*

- **C.C. Chang, Ph.D.**, Michigan State University, "Development of multipotent stem cells by chemical supplementations in culture medium"
- **Deborah Gumucio, Ph.D.**, University of Michigan, "Gastric progenitor cells: A link to gastric cancer?"
- **Pamela G. Robey, Ph.D.**, NIDCR, NIH, "Skeletal stem cells: The cause and the cure"
- **Gary Hammer, MD, Ph.D**., University of Michigan, "Adrenal stem/progenitor cells: Implications for cancer"

10:20 – 11:50 a.m. **Stem Cell Therapy – Session II (BanqB)**
Moderator: *David Felten, M.D., Ph.D., Vice President of Research and Medical Director, Beaumont Research Institute, Beaumont Hospital*
Heart, Lung, and Blood Diseases

- **Thomas Bauer, Ph.D.**, NCI, NIH, "Update on hematopoietic stem cell gene therapy"
- **Shannon McKinney-Freeman, Ph.D.**, Harvard Medical School and Children's Hospital, "Making blood from embryonic stem cells: A sanguine future"

Developmental Diseases

- **Mark Furth, Ph.D.**, Wake Forest Institute for Regenerative Medicine, "Potential of amniotic fluid-derived stem cells for regenerative medicine"
- **Jack Mosher, Ph.D.**, University of Michigan, "Neural crest stem cells in development, disease, and therapy"

12:00 – 1:00 p.m. Lunch (Oakland Room & Gold Room)

1:00-2:00 p.m.**Panel- Stem Cell Ethics/Political Implications (Banq A)**
Welcome by Ananias Diokno , M.D., Chief Medical Officer, Beaumont Hospital
Moderator: *Ken Hightower, Ph.D., Dean, School of Health Sciences, Oakland University*

- **Graham Parker, Ph.D.**, Wayne State University Children's Hospital of Michigan, "Stem cells and clinical practice: Pragmatics before therapeutics"
- **Christine Gorka, Ph.D.**, Providence Hospital, "Stem cell disparities: Addressing ethical concerns around justice"
- **Representative Andy Meisner,** D-Michigan

1:00-2:00 p.m. **Young Investigator Oral Presentations (Banq B)**
Moderator: *Ken Mitton, Ph.D., Associate Professor, Eye Research Institute, Oakland University*

- **Jill Slater,** Wayne State University, "Stress enzyme activation tips the balance from pluripotency to differentiation in embryonic and trophoblast stem cells"
- **Chong Chen**, University of Michigan, "Tsc-mTOR pathway maintains quiescence and function of hematopoietic stem cells by repressing production of reactive oxygen species"
- **Sichang Zhou,** Wayne State University, "Long-term effects of stress on embryonic and trophoblast stem cells is through global changes in effectors of differentiation"
- **Asamoah Bosomtwi,** Henry Ford Hospital and Oakland University, "Quantitative Detection of Microvascular Density Changes after cell therapy in Stroke rats"

SATURDAY, MAY 10 CONTINUED

2:00 – 3:30 pm **Drug Design/Development and Biotechnology– Session (Banq A)**
Moderator: *Ibrahim Ibrahim, M.D., M.P.H, Ph.D. Director, Urology Outcomes Research, Beaumont Hospitals*

- **Peter Sartipy, Ph.D.**, Cellartis, Sweden, "Human embryonic stem cells for drug discovery"
- **Barbara Tigges, Ph.D.**, BioE, Inc. Research and Development,"Umbilical cord blood-derived multi-lineage progenitor cells (MLPC), a model for tissue engineering anddrug discovery"
- **William Fairbrother, Ph.D.,** Brown University "High-throughput Biochemical profiling of DNA/protein complexes at Oct4 bound genomic regions"

2:00 – 3:30 p.m. **Tissue Engineering– Session (Banq B)**
Moderator: *Donggang Yao, Ph.D., Associate Professor, Georgia Institute of Technology*

- **Shuichi Takayama, Ph.D.**, University of Michigan, "Microfluidics for stem cell biology and therapy"
- **Donggang Yao, Ph.D.**, Georgia Institute of Technology, "Fabrication of functional porous structures for tissue engineering"
- **Luis Villa Diaz, Ph.D.**, University of Michigan, "Use of synthetic polymers for the culture of hESCs"
- **Thomas Diekwisch, D.M.D., Ph.D.**, University of Illinois at Chicago, "Dental stem cells and periodontal regeneration"

3:30 – 4:00 p.m. Coffee Break
4:00 – 5:30 p.m. **Stem Cell Biology - Session III (Banq A)** ***Moderator:*** *Sue O'Shea, Ph.D., Professor of Cell & Developmental Biology, University of Michigan*

- **Dean Tantin, Ph.D.**, University of Utah, "The homologous transcription factors Oct1 and Oct4 are signal integrators and regulators of stemness"
- **José Garcia Perez, Ph.D.**, University of Michigan, "LINE-1 retrotransposition in human embryonic stem cells"
- **Vilma Yuzbasiyan-Gurkan, Ph.D.,** Michigan State University "Adipose Tissue as a Promising Source of Mesenchymal Stem Cells: Studies in a Canine Model"

6:30 – 7:30 p.m. **Welcome (Banq A & B)** *by G. Rasul Chaudhry Ph.D.,and Virinder K. Moudgil, Ph.D., Sr.VP of Academic Affairs and Provost, Oakland University*
Introduction of Keynote Speaker *by Pamela Robey, Ph.D. NIH*

- **Andras Nagy, Ph.D.**,Mount Sinai Hospital, Toronto, "Mouse Genetics: The changing landscape"

7:30 – 9:00 p.m. Dinner/Closing Remarks(Banq A&B)
SUNDAY, MAY 11
Location: Oakland Room, Oakland Center
8:00– 10:00 a.m. Continental Breakfast, Business Meeting and Closing Remarks
10:05 a.m. Conference Adjournment

BIOSKETCHES

&

PRESENTATIONS

SUMMARIES

Kameswara Rao Badri, Ph.D., is presently working as Assistant Professor (Research) at Wayne State University. I received my Ph.D in Biochemistry from Sri Venkateswara University, India. I started my research career as postdoctoral fellow in 2001 at University of Delaware and in 2002 moved to Wayne State University. In 2004 I met Prof. Lucia Schuger and I developed interest in TIPs, joined her lab and started working on the role of TIPs in mesenchymal cell lineage differentiation.

Presentation Summary: Bronchial smooth muscle develops from local mesenchymal cell precursors. Mechanical tension plays an important role in initiating bronchial myogenesis. While studying the effects of mechanical tension we identified TIP-1 and TIP-3 (Tension Induced/inhibited Protein-1 and 3) in mouse lung embryonic mesenchymal cells. TIP-1 was induced by stretching and induced myogenesis. On the contrary, TIP-3 was suppressed by stretch. In non-stretched lung embryonic mesenchymal cells TIP-3 induced adipogenesis. Here we report that the TIP family comprises eight isoforms, all bearing a SANT domain, characteristic of histone modifying enzymatic complexes. TIPs have SANT-dependent, p300-mediated histone acetyltransferase (HAT) activity. Furthermore we reveal the involvement of TIP-6 in the induction of PPAR□2-mediated adipogenesis.

Thomas R. Bauer, Jr., Ph.D., is a staff scientist at the National Cancer Institute, NIH, in Bethesda, Maryland. Dr. Bauer received his B.S. in microbiology from the University of Notre Dame in 1985, and his Ph.D. in microbiology and immunology from the University of Miami (Florida) in 1992. Dr. Bauer was a post-doctoral fellow with Dr. Dennis Hickstein in the Department of Hematology at the University of Washington in Seattle, Washington and was supported by an National Research Service Award from the NIH. Dr. Bauer was then appointed to the faculty of UW as an instructor in 1998. He joined the Experimental Transplantation and Immunology branch of the NCI in 2001. Working in the field of gene therapy since 1992, Dr. Bauer was one of the earliest members of the American Society of Gene Therapy. Dr. Bauer was co-investigator for the clinical gene therapy trial "*Retrovirus-Mediated Transfer of the cDNA for Human CD18 into Peripheral Blood Repopulating Cells of Patients with Leukocyte Adherence Deficiency"*. Since 1999, Dr. Bauer has focused on the canine model of leukocyte adhesion deficiency to develop new therapeutic applications.

Presentation Summary: Hematopoietic stem cells (HSCs) have emerged as attractive gene therapy targets for a wide range of diseases, including cancer, AIDS, and inborn genetic disorders. Gene therapy of several genetic diseases, including SCID-ADA, SCID-X1, and CGD, have achieved success, but also setbacks in the form of leukemias from insertional activation by the gammaretroviral vectors used to treat patients in these trials. Improved vectors based on lentiviruses and foamy viruses are currently being investigated in the treatment of several diseases. In our lab, we have successfully treated several dogs with the genetic disease leukocyte adhesion deficiency using foamy viral vectors. Continued development of these and other vector systems along with new technologies such as site-specific gene correction are likely to lead to new strategies for gene therapy of human genetic diseases using hematopoietic stem cells.

Asamoah Bosomtwi, M.S., received his BSc degree from University of Cape Coast, Ghana in 1994; Masters in radiation science from University of Botswana in 1998; MS in condense mater physics from University of Kentucky in 2004, and just finished defending his PhD dissertation at Oakland University. Title of his dissertation is "Quantitative Determination of Microvasculature after Neurovascular Diseases in Rat Using MRI" supervised by Prof. Micheal Chopp and Dr. Quan Jiang. He did his research at neurology department NMR- lab in Henry Ford hospital.

Presentation Summary: This presentation will cover the recent investigation of vascular remodeling after stroke using MRI microvascular density (MVD) measurement and gold standard immuno-histochemistry staining to demonstrate MRI MVD detect the microvascular status of brain tissue with different ischemic damage. The MVD measured by MRI was highly correlated with histological measures of MVD. Our data demonstrate that MRI MVD measurement can quantitatively evaluate microvascular remodeling after stroke.

Jaime M. Brozowski, B.S., graduated with honors from Lyman Briggs College at Michigan State University with a Bachelor of Science degree in human biology in 2007. She is currently a graduate student at Oakland University studying cellular and molecular biology with specialty in embryonic (ESC) and human umbilical cord stem (CBSC) cell research. Her current research at Oakland University focuses on the regulation of stemness and differentiation of ESCs and CBSCs, as well as toxicological studies investigating embryotoxicity, fetotoxicity, and teratogenicity of gold nanoparticles, various polychlorinated biphenyls, and pesticides, which may interfere with early developmental processes.

Presentation Summary: Recent advancements in stem cell research provide promising ways and sources to isolate stem cells with vast proliferative and differentiation abilities. This workshop focuses on the specific isolation and culture of embryonic stem cells and human umbilical cord stem cells.

Michael B. Chancellor, M.D., is the director of neurourology program in the Department of Urology at the William Beaumont Hospital in Royal Oak, Michigan. He earned his medical degree at the Medical College of Wisconsin in Milwaukee and then completed his internship in surgery and his residency in urology at the University of Michigan in Ann Arbor and his fellowship in neurourology and female urology at Columbia University College of Physicians and Surgeons in New York. Dr Chancellor has been featured in several publications, and he was chosen as the director of National Institutes of Health–funded clinical trials for women focusing on stress urinary incontinence and pelvic prolapse. He is now concentrating his efforts on translational research, including the use of muscle-derived stem cells to treat stress urinary incontinence.

Presentation Summary: Approximately 13 million Americans are living with urinary incontinence -- a condition that causes the bladder to leak urine. It's nearly twice as common in women, and many don't seek help. Surgical slings, pills and exercises are a few common treatments, but researchers say stem cell therapy could offer new hope to people looking to live a life free of embarrassment. Building on the pioneering work of Drs Michael Chancellor and Johnny Huard, and under a license agreement with the University of Pittsburgh, Cook MyoSite Inc., is developing technology involving the use of autologous muscle-derived cells for treating urinary incontinence and other diseases. These autologous cells - cells taken from the patient - may also have the ability to repair other damaged tissues.

Chia-Cheng Chang, Ph.D., received his Ph.D. in Genetics from the University of Missouri in 1971. After postdoctoral works on somatic cell genetics at Oak Ridge National Laboratory and the University of Michigan in 1974, he came to Michigan State University, where he spent his entire academic career until present. Dr. Chang's research areas include stem cell biology and mechanisms of mutagenesis and carcinogenesis, especially the role of cell-cell communication in tumor promotion and the role of stem cells in tumorigenesis. Since 1987, he published many papers on human adult stem cells, i.e. kidney, breast epithelial, pancreatic, liver, gastric and adipose-derived mesenchymal stem cells (MSCs). Among the notable discoveries are the finding that a breast epithelial stem cell type, as target cells for breast carcinogenesis, was more susceptible to telomerase activation, immortalization and neoplastic transformation, the development of a novel cell culture method for adipose-derived MSCs with accelerated growth and extended lifespan, and the demonstration that many human adult stem cells expressed the embryonic stem cell marker, Oct-4. His general approach to developing adult stem cells is the chemical modification of cell culture condition to modulate the expression of few pivotal genes.

Presentation Summary: At present, there are 3 major approaches to developing stem cells for clinical applications, i.e. 1). Heterologous or patient-specific embryonic stem cells; 2). Induced pleuripotent stem cells (iPCs) by gene transfer; and 3). Chemical modification of culture condition to grow multipotent adult stem cells. My presentation will focus on the third approach. In literature, several chemicals have been reported to maintain the growth of multipotent stem cells by modulation of the expression of few pivotal genes. I will show the usefulness of N-acetyl-L-cysteine and L-ascorbic acid 2-phosphate in modification of cellular redox and the growth of several adult stem cells.

Chong Chen, B.S., is a Cell and Developmental Biology graduate student at the University of Michigan. He has independently initiated the studies of hematopoietic stem cells in Dr. Pan Zheng's laboratory in the Department of Surgery and Pathology. He has made a very interesting observation on the roles of Tsc-mTOR pathway on hematopoietic stem cells.

Presentation Summary: Chen has observed the roles of Tsc-mTOR pathway on hematopoietic stem cells and has demonstrated that reactive oxygen species is downstream of mTOR to maintain the stemness.

Michael Chopp, Ph.D., is Distinguished Professor of Physics at Oakland University. He also joined Henry Ford Hospital in 1983, was appointed Vice Chairman for Research of the Department of Neurology in 1991, Scientific Director of the Henry Ford Neuroscience Institute in 1999, and is the Zoltan J. Kovacs Chair in Neuroscience Research. He received his MS and PhD in Mathematical and Solid State Physics from New York University. Dr. Chopp's research has primarily focused on: 1) the cellular and molecular biology of ischemic cell injury, 2) the pathophysiology of stroke, 3) combination thrombolytic and neuro and vascular protective therapies for stroke, 4) mechanisms of neuroprotection, 5) cell-based and pharmacological neuro-restorative therapies for stroke, traumatic brain injury and neurodegenerative disease, 6) molecular and cellular mechanisms underlying neurogenesis and angiogenesis and the induction of brain and spinal cord plasticity leading to functional and behavioral recovery after neural injury, 7) treatment of glioma, 8) and magnetic resonance imaging. Dr. Chopp has more than 412 peer reviewed publications. He has chaired National Institutes of Health (NIH) study sections and has often served as a consultant to government agencies, the U.S. National Institutes of Health, and the pharmaceutical industry.

Presentation Summary: I will describe our data on the multiple therapeutic targets of cell-based treatment of neurological disease, including stroke. We have treated cerebral infarction with a number of different cell types; however, we will focus the present data on treatment with bone marrow mesenchymal cells (MSCs). All the animals are subjected to a battery of neurological outcome measures. Our data show that there is significant and persistent therapeutic benefit when MSCs are administered via a vascular route 1, 7 or 30 days after stroke onset, with benefit out to a year post treatment. Cell therapy stimulates a tapestry of restorative events in the brain and spinal cord, and catalyze the expression of a variety of

trophic factors, chemokines and angiogenic proteins by parenchymal cells which enhance neurogenesis, angiogenesis, and synaptogenesis and induce rewiring of neurites in the spinal cord and brain.

Mick Perez-Cruet, M.D., M.S. is a neurosurgeon who practices with a multi-specialty group in Detroit, Michigan. He received a B.A. degree from Grinnell College in Iowa in 1983 and then pursued a Masters in Chemistry from the University of South Florida in Tampa. He received his MD degree in 1991 under an Air Force Health Professions Scholarship from Tufts University in Boston. He completed his neurosurgical residency at Baylor College of Medicine. As a Major in the Air Force he served as Vice-Chairman of the Department of Neurosurgery at Wilford Hall Medical Center, Lackland Air Force Base, San Antonio, Texas. He then pursued a spine fellowship in Reconstructive and Minimally Invasive Spine Surgery at Rush University and the Chicago Institute of Neurosurgery and Neuroresearch. He is currently an adjunct Associate Professor at Oakland University, Rochester, Michigan.Dr. Perez-Cruet is best known for his pioneering work in minimally invasive spine surgery. He is the Director of Minimally Invasive Spine Surgery and Spine Program at the Michigan Head and Spine. He has edited three text books (Outpatient Spinal Surgery and An Anatomical Approach to Minimally Invasive Spine Surgery, and Minimally Invasive Spinal Fusion: Techniques and Operative Nuances), multiple chapters and peer -review manuscripts. His research interests are in regenerative spinal stem cell technologies and innovative minimally invasive spinal techniques.

Presentation Summary: This session will focus on the clinical application of stem cell therapy. Participants will learn current cutting edge research that is being conducted to treat degenerative spinal disorders, eye diseases, multiple sclerosis, and cerebral strokes from leaders in the field. Participants will be able to appreciate a number of disorders that can be treated using stem cell therapies and understand the process of applying basic science stem cell research to the clinical arena.

Luis Villa Diaz, Ph.D., was born in 1971, in Orizaba city, in the State of Veracruz, in Mexico. His education was done in Orizaba city in Marist school. In 1994, he got his Doctor degree in Veterinarian Medicine from the Universidad Veracruzana, which is the Veracruz State University. He specialized in reproduction of large animals, such as bovine and equine, and practice as DVM for 3 years. Then in 1999, he obtained my Master in Applied Science, with major in Physiology of Reproduction from Lincoln University, Canterbury, New Zealand. His research focused on the secretion of the luteinizing hormone in red deer hinds. In 2004, he got my PhD degree with major in Biology of Reproduction, from Kobe University, Kobe, Japan. He researched on the role of the MAPK family on porcine oocyte maturation. From 2004 to 2007, he worked in the Medical School of the University of Michigan, in the Ob/Gyn Department, with Dr. Gary D. Smith researching in human embryonic stem cells. The research focused in culture optimization, cryopreservation, microfluidics, and the use of synthetic polymers as substrates for hESC culture. Since October 2007, he has worked in the Dental School of UM, in the Biologic and Material Science Department, with Dr. Paul Krebsbach. His research focused on the interconnection between extracellular matrices and hESCs and hESC-derived mesenchymal stem cells and in the immobilization of growth factors and proteins in 2D and 3D substrates to use in bone regeneration from hESC-MSCs.

Presentation Summary: Current culture methods of hESCs have pitfalls that need to be address in order for these cells to be considered therapeutic grade. With the use of synthetic polymers as substrates matrix we pretend to get closer to the objective of a full-defined culture. After screening several synthetic polymers with different chemical and mechanical characteristics, one of them, the PMEDSAH, was found to support long-term culture of hESCs. Full characterization of hESCs cultured on PMEDSAH showed, that they retain normal karyotype, pluripotency, self-renewal, and ability to differentiate *in vivo* and *in vitro*.

Thomas G.H. Diekwisch, D.M.D., Ph.D. (Sc.), Ph.D. (Phil.), was born in Bielefeld, Germany. He holds degrees in dental medicine (1986, D.M.D.), a Ph.D. in Anatomy (1988, "*summa cum laude*"), and a Ph.D. in philosophy (2005, "*magna cum laude*"), all from the Philipps-University of Marburg. From 1986-1990, Dr. Diekwisch worked as a lecturer, clinical instructor, and research associate in the Departments of Anatomy and Periodontics at the Philipps-University. In 1990, he became a postdoctoral fellow in craniofacial biology at the University of Southern California. In 1994, he joined the faculty of Baylor College of Dentistry in Dallas, TX, where he created an award-winning community science education outreach program entitled "Habitat for Science". In 2001, Dr. Diekwisch was recruited to the University of Illinois at Chicago to become the first Director of the Brodie Laboratory for Craniofacial Genetics and the Allan G. Brodie Endowed Chair. Two years later, he was appointed Professor and Head of the Department of Oral Biology at UIC. Besides Oral Biology, Dr. Diekwisch holds appointments in Anatomy and Cell Biology, Bioengineering, Orthodontics and Periodontics. Diekwisch discovered and characterized a gene, CP27 that plays an important role in craniofacial development. Other research areas include the development and evolution of tooth enamel and periodontal tissues as well as craniofacial tissue engineering.

Presentation Summary: On their path from the free regions of the neural fold to their target tissues, neural crest cells are subjected to a multitude of factors and microenvironments presented by local substrates and surface conditions along their migratory path. The majority of these signals and structural cues are provided by a unique protein environment

surrounding living cells, the extracellular matrix. Together, the studies presented here illustrate the importance of an extracellular matrix/stem cells interplay in craniofacial tissue engineering.

Kimberly Drenser, M.D., Ph.D., is a viteroretinal surgeon and the Director of Ophthalmic Research with Associated Retinal Consultants and an Assistant Professor at Oakland University, Eye Research Institute, Michigan, USA. Dr,. Drenser completed her medical degree and Ph.D. at the University of Florida and created a ribozyme for the treatment of autosomal dominant retinitis pigmentosa. The animal studies demonstrated potential use of a gene therapy for treatment. She went on to complete a residency in Ophthalmology at the Jules Stein Eye Institute, followed by fellowship training in vitreoretinal surgery with Associated Retinal Consultants. She currently is in clinical practice and specializes in diseases of the vitreous and retina with an emphasis on pediatric diseases. Dr. Drenser continues basic research with specific interest in pediatric vitreoretinal diseases and retinal development. Specifically, she has analyzed the biochemical constituents of the developing vitreous in infants and children with various vitreoretinopathies. This research has an emphasis on vascular endothelial growth factors (VEGF) and associated angiogenic factors. Dr. Drener has a particular interest in how these factors effect management of retinal diseases and how current pharmaceutical and surgical interventions may be improved. These studies overlap with ongoing research regarding pathways involved in retinal development. In particular, Dr. Drenser studies the Wnt receptor: β-catenin signal transduction pathway. This pathway is implicated in a number or pediatric vitreoretinopathies and its evaluation may improve the current understanding of retinal differentiation and maturation.

Presentation Summary: The retina is one of the last tissues to develop in the human. A number of disease states exist which present a glimpse into the normal and abnormal events of retinal development. Retinopathy of Prematurity is a disease that occurs in infants born before the retina is complete, providing direct evaluation of a developing retina. Inherited vitreoretinopathies share common characteristics of ROP but are due to abnormalities in genes regulating vasculogenesis and retinal maturation. Understanding the events that signal normal development and studying diseases characterized by abnormal development may lead to the successful manipulation and regulation of stem cells in eye diseases.

Gregory Dressler, Ph.D., is a Collegiate Professor of Pathology Research at the University of Michigan Medical School. He received a B.S.E. and a Ph.D from the University of Pennsylvania and was a post-doctoral fellow in the laboratory of Peter Gruss at the Max Planck Institute of Biophysical Chemistry in Goettingen. Upon returning to the US, he was a Senior Staff Fellow at the National Institutes of Health in Bethesda before coming to Michigan. For the past 15 years, Dr. Dressler's has lab has studied the role of transcription factors and cell signaling pathways in renal development and disease. The lab has made numerous discoveries including: the Pax2 gene, the function of GDNF/c-ret in kidney development and chemotaxis, the function of KCP, the first enhancer of BMP signaling, in renal fibrosis, and the function of the epigenetic co-factor PTIP in histone methylation.

Presentation Summary: Stem cells and embryonic development are intricately linked. As tissues are specified, cells become increasingly restricted in their potency yet must still undergo many rounds of cell division. Using the kidney as a model system, I will discuss some of the genes and proteins that control early cell lineage events and renewal of kidney epithelial stem cells. How such factors impact the genome through interactions with epigenetic regulators will be discussed.

William Fairbrother, Ph.D., attended graduate school at Columbia University working in the Chasin lab where he learned library/selection approaches in somatic cell genetic systems. He then moved to Boston for a postdoc at MIT in Phil Sharp's Lab. His interest in computational methods brought him in close contact with Chris Burge's Lab and as a collaborative project he developed the first computational screen for enhancer sequences. Dr. Fairbrother is currently an assistant professor in the MCB Department at Brown University. His lab uses a combination of computational biology and high throughput genomics techniques to identify functional elements in the genome. Recently he has applied these techniques to the study of the Oct4 transcription factor. He is currently mapping how this important player in the maintenance of pluripotency binds DNA and regulated its target.

Presentation Summary: We have developed a high throughput interaction assay that determines the binding specificity of the transcription factor, Oct4. This work starts with the output of ChIP-chip assay and searches within these vivo binding regions for precise sites of Oct4 binding. Not only are these precise sites of Oct4 identified, we also learn how Oct4 is binding (monomer or multimer or as a larger complex). We demonstrate how we can reliably identify the binding motifs of Oct4 sequences and that the consensus ATGCAAAT sequence is not the strongest binding site. Part of the larger interest in mapping Oct4 binding sites is to explain how the context around Oct4 binding sites, in some cases, causes Oct4 appears to act positively on transcription and, in other cases, negatively on transcription. To achieve this we attempt to characterize the factors that bind near Oct4 to make the core transcriptional regulatory circuitry of ES cell specific genes.

Chris Fecek, B.S., graduated from Oakland University in 2008 with a Bachelor of Science in Biology and a minor in Exercise Science. Since 2006, he has been working on stem cell research in Dr. Rasul Chaudhry's laboratory at Oakland University. Chris's major research focus involves investigating tissue replacement therapy using ESC derivatives. Currently, Chris is working on a project in collaboration with Providence Hospital exploring the potential for chondrogenic derivatives to replace the nucleus pulposus in degenerated intervertebral disc tissue. He has also worked on projects involving tissue engineering, the effects of nanogold on ESCs, and the isolation of human mesenchymal stem cells from umbilical cord blood.

Presentation Summary: This workshop will briefly cover the basic stem cell line maintenance and will address stem cell differentiation, including: the Hanging drop method, importance of differentiation medium, isolation of differentiated cell types, identification and characterization of primary cells, and culturing of primary cells.

Shannon McKinney-Freeman, Ph.D., is a senior postdoctoral fellow in the laboratory of George Daley at Children's Hospital Boston and Harvard Medical School. She is currently funded by a K01 from the NIH/NIDDK and an American Society of Hematology Scholar Award. During her tenure in the Daley lab she has received support as an Eleanor and Miles Shore Scholar and via a postdoctoral fellowship from the American Cancer Society. Her research in the Daley lab focuses on the phenotypic, functional and molecular characterization of embryonic stem cell-derived hematopoietic stem cells (HSC) and developmental HSC compartments, as well as the role of the *Cdx* gene family in HSC development. Prior to joining the Daley lab, Dr. McKinney-Freeman completed her Ph.D. in the laboratory of Margaret Goodell at Baylor College of Medicine in Houston, TX, where she determined that skeletal muscle-derived HSC potential was the result of itinerant bone marrow-derived HSC, rather than trans-differentiating myogenic stem cells. Dr. McKinney-Freeman completed her B.A.s in Biology and Chemistry at Ripon College in Ripon, WI and hails from Spokane, WA.

Presentation Summary: Embryonic stem cells (ESC) represent a potential source of transplantable hematopoietic stem cells (HSC). A major goal of our laboratory is the derivation of potent HSC from human ESC that could be exploited in the clinic to treat hematopoietic malignancies and genetic disease. There are multiple projects ongoing in the laboratory that all contribute towards the realization of this goal: 1) the characterization of ESC-derived HSC (ESC-HSC) generated from murine ESC, 2) the identification and characterization of exogenous factors that promote the specification of blood progenitors in differentiating murine ESC, and 3) the derivation of patient specific human iPS cell lines. This presentation will be an overview of recent advances within the laboratory towards each of these projects.

Mark Furth, Ph.D., is a molecular biologist with experience both in academics and the biopharmaceutical industry. Dr. Furth joined the Wake Forest Institute for Regenerative Medicine in March 2005, where he is Science Officer and helps to direct the Institute's stem cell programs. He has served at senior levels, including Chief Scientific Officer and Chief Executive Officer, in several biotechnology companies in the fields of regenerative medicine, genomics, and drug discovery, and was Vice President of Molecular Sciences at the Glaxo Research Institute (now GlaxoSmithKline). Previously, he worked in oncogene research as a faculty member at the Memorial Sloan-Kettering Cancer Center and, concurrently, the Cornell University Graduate School of Medical Sciences. Dr. Furth obtained his B.A. at Harvard University and his Ph.D. in Molecular Biology from the University of Wisconsin-Madison. His postdoctoral training was carried out at the Medical Research Council Laboratory of Molecular Biology, Cambridge, UK, with Nobel laureate Sydney Brenner, and at the National Cancer Institute with Edward Scolnick.

Presentation Summary: Numerous applications in tissue engineering and regenerative medicine depend on the availability of appropriate stem and progenitor cells. Programs at the Wake Forest Institute for Regenerative Medicine utilize multiple sources, including autologous cells and allogeneic stem/progenitor cells. Amniotic fluid-derived stem (AFS) cells represent a potentially valuable new source. The AFS cells and comparable cells from placenta can expand extensively and yield differentiated progeny with markers representing each of the three embryonic germ layers. However, unlike embryonic stem cells they do not form teratomas. The relative advantages of AFS cells, induced pluripotent stem (iPS) cells, and other stem/progenitor populations being developed at the Institute will be discussed.

Christine Gorka, M.S., M.A., is the Ethicist for the St. John Health System, Detroit, MI, where she is responsible for the overall planning, operation, and evaluation of ethics education and integration. Christine has a extensive research background that spans more than 24 years, 15 years as a Discovery Research scientist in the pharmaceutical industry (Monsanto Corporate Research/ Searle/ Pharmacia/ Pfizer) studying a wide variety of diseases including cardiovascular, metabolic, cancer, and inflammatory. She is a graduate of Eastern Illinois University in Charleston, Ill, where she received Bachelor of Science (botany, environmental biology) and a Master of Science (environmental biology). In 2004, Christine completed her Master of Arts degree in theology at Aquinas Institute of Theology in St. Louis, Mo. In 2007, she finished course work and passed her comprehensive exams toward her doctorate in ethics from St. Louis University's Center for Health Care Ethics. She is currently in the dissertation phase. Christine has conducted research in the health care sciences, has published in scientific journals, and authored several clinical book chapters and abstracts. Her research interests are focused on the effect marginalization (e.g., gender, racial, socio-economic) has on health care access, utilization, and outcomes.

Presentation Summary: For many, the only ethical concern regarding stem cell (SC) research involves the moral question around using human embryos. However, there exist serious justice concerns relating to these promising therapies regardless of the stem cell source. New technologies are expensive. Unless addressed, economic barriers will make SC therapies inaccessible to some patient populations. In addition, there is growing recognition that ethnicity may also limit access and minorities may not benefit from this technology. Since SC therapies will come from designed cell banks,

disparities can be minimized in the bank construction. This presentation will address the ethical mandate of distributive justice by exploring what barriers (economic and biological) need to be considered as stem cell research moves forward.

Theresa E Gratsch, Ph.D., is a postdoctoral fellow in Dr. Sue O'Shea's laboratory at the University of Michgan Medical School. Her current position is Research Laboratory Specialist Senior in the department of Cell and Developmental Biology.

Presentation Summary: This workshop focuses on the specific isolation and culture of human embryonic stem cells.

Deborah Gumucio, Ph.D., completed her B.A., M.S. and Ph.D. degrees as well as postdoctoral studies (with Francis Collins) at the University of Michigan. She was appointed to the faculty in 1991 (then Department of Anatomy), and was promoted to Associate professor in 1996 and full Professor in 2002 (now Department of Cell and Developmental Biology). She has maintained an active research laboratory that has been continuously well-funded since 1987. Her investigations have focused on mouse models of human disease including: a) globin gene regulation and its application to Sickle cell disease and beta thalassemia; b) molecular etiology of familial Mediterranean fever (a disease of the innate immue system); and c) organogenesis of the intestine and stomach (with relevance to inflammatory bowel disease, celiac disease, intestinal metaplasia and gastric cancer).

Dr. Gumucio is the Director of the Michigan Center for Organogenesis, founded in 1995. This Center includes >100 faculty members in 28 departments of five schools and colleges of the UM. The goal of the Center is to increase communication and collaboration among clinical, basic and applied scientists engaged in Organogenesis research. An integral arm of the Center is the Organogenesis Training Program, which has been continuously funded for 11 years. Last year, this Program was recognized as the #1 NICHD-sponsored Training Program nationally. The novel structure includes a public outreach arm that uses beautiful photographic images taken during scientific research to educate the public about scientific issues. This program, called Bioartography, is a unique blend of science, photography and art; profits from images sold at the Ann Arbor Art Fair or via the web *(www.bioartography.com)* are used to augment the Training Program.

Presentation Summary: Epithelial stem cells in the stomach are responsible for the constant renewal of the epithelium and the generation of multiple gastric cell lineages that populate the gastric glands. However, gastric stem or progenitor cells have not been well-characterized due to the lack of specific markers that permit their prospective recognition. We identified an intestinal promoter (villin gene) that is active in a rare subpopulation of gastric epithelial cells and investigated whether these cells possess multi-lineage potential. A marked allele of the endogenous mouse villin locus was used to visualize single β-galactosidase positive cells located in the stem cell zone of a subset of gastric glands. Our studies demonstrate that these rare villin-marked epithelial cells are highly quiescent, but that interferon gamma administration results in their robust amplification. Lineage tracing studies confirm that these cells are multipotential and give rise to all gastric lineages of the gland. Recent microarray analyses of the isolated cells have resulted in the identification of additional potential markers for gastric stem cells. The ability to prospectively identify and manipulate gastric progenitors *in situ* represents a major step forward in gastric stem cell biology and has implications for gastric cancer.

Vilma Yuzbasiyan-Gurkan, Ph.D., obtained her bachelor degree from Vassar College majoring in Biochemistry in 1978. She continued her education in a doctoral program in Biochemistry with a strong medical component at the University of Istanbul focusing on copper and zinc handling and their disorders in pediatric patients. Upon completion of her degree, she joined the University of Michigan Department of Human Genetics as a post-doctoral fellow and focused on Wilson disease. While working on a canine model of Wilson disease, canine hereditary copper toxicosis, she started to develop mapping tools for the canine genome. This led to the identification of a novel locus for copper transport. She has since been working on comparative genetics. Her research group has recently focused on characterization of resident stem cells from tissues in the adult canine, especially mesenchymal stem cells, and studying them both for their reparative potential and for probing them to understand mechanisms of tumorigenesis. Her group has recently provided the first report of the isolation and characterization of mesenchymal stem cells from the dog adipose tissue.

Presentation Summary: We report the isolation and extensive characterization of mesenchymal stem cells from canine adipose tissue. Pluripotency of these cells was documented by differentiation into adiposite, chodrocyte and osteoblasts. The canine adipose derived mesencymal stem cells (cAD-MSCs) displayed anchorage independent growth in soft agar, and their colony forming efficiency in plastic was comparable with human counterparts. The cAD-MSCs expressed genes associated with pluripotency while their differentiated progeny expressed appropriate lineage specific genes. The optimization of growth and differentiation of cAD-MSCs should facilitate future stem cell-based reparative and regenerative studies in dogs. The dog is a promising biomedical model which is suitable for evaluation of novel therapies such as those employing stem cells in experimental and in spontaneous disease settings.

Gary Hammer, M.D., Ph.D., is a highly respected researcher, clinician, teacher and mentor. He has served in leadership positions on numerous committees for the Endocrine Society and sits on the editorial board of Molecular Endocrinology receiving the 2005 Outstanding Reviewer Award. His most notable awards include the 1999 Endocrine Society Senior Fellow Award and the 2005 Jerome Conn Award from the University of Michigan, the top award given by the Department of Medicine to a junior faculty. In the same year, co-incident with tenure, Dr. Hammer was installed as the Millie Schembechler Professor of Adrenal Cancer and named Director of the Endocrine Oncology Program in the Cancer Center which incorporates diagnosis, treatment and research of adrenal cancer.

Presentation Summary: The long range objective of my laboratory is to understand the cellular and molecular mechanisms by which signaling pathways and downstream transcription factors coordinate the specification of adrenocortical cells within the adrenal gland in health and disease. Recent efforts examine the hypothesis that IGF and Wnt/beta-catenin signaling maintains the functional capacity of the adrenal cortex though the regulation of undifferentiated adrenocortical cell fate. Dysregulation of this system is predicted to result in abnormal adrenocortical growth and/or differentiation. Using cellular systems, mouse models together with genomic approaches with mouse and human adrenocortical carcinoma (ACC) samples, we aim to characterize the stem/progenitor cells of the adrenal cortex and uncover the mechanisms by which these cells are regulated by various signaling pathways in normal adrenal growth maintenance and cancer.

In the adrenal cortex, IGF and Wnt/beta-catenin signaling are restricted to the subcapsular region. While these subcapsular undifferentiated adrenocortical cells are known to migrate centripetally into the cortical zones of the gland to populate the three zones of the adrenal cortex, the molecular mechanism underlying role of these cells in tissue homeostasis is poorly understood. We present data that support a role of IGF and Wnt/beta-catenin signaling in the self-renewal and multipotent properties of these adrenocortical cells in vivo. We also characterize mechanisms by which gain of IGF and Wnt/beta-catenin signaling participate in the development of ACC. ACC is an incredibly rare and routinely fatal disease with few effective treatments. Understanding the role of the signaling pathways in adrenocortical cell fate will lay essential groundwork for future therapies that target this pathway and downstream genes that are found in the course of these studies to participate in adrenocortical stem/progenitor cell biology.

Ales Hampl, D.V.M., Ph.D., obtained his D.V.M. at Brno Veterinary University in the Czech Republic, and conitnued to persue his Ph.D. in Animal Physiology and Genetics at the Academy of Sciences of the Czech Republic. He completed his postdoctoral fellowship at The Jackson Laboratory in Maine, USA with John Eppig, as he advisor. In 1995, he was appointed Staff scientist at the Academy of Sciences of the Czech Republic. Since 2005, he serves as the Head of the Department of Molecular Embryology at the Institute of Experimental Medicine in the Czech RepulicCR. He is also an Associate Professor in the Department of Biology and Faculty of Medicine at Masaryk University in the Czech Republic. He acts as a PI in international projects focusing at various aspects of biology of human embryonic stem cells, such as global International Stem Cell Initiative and EU project Platforms for Biomedical Discovery with Human ES Cells. He is the member of the Standards Committee of the ISSCR. His major research interest is the role of the cell cycle regulators in developing oocytes and cells of embryonal origin, as well as derivation, propagation, and differentiation of human embryonic stem cells.

Presentation Summary: Although it was not first recognized, recent studies have clearly shown that genetic information of human embryonic stem cells (hESC) that are propagated in culture is rather vulnerable and undergoes changes at various levels. Molecular mechanisms, by which alterations to the genome of hESC develop, as well as the biological outcomes of such genetic changes are not yet understood. Here we show that undifferentiated hESC has established at least certain segments of molecular circuitries that are effective in sensing damage to DNA and in executing an appropriate response to it. We also show that generation of gross chromosomal mutations, amplifications and translocations, which are frequently generated in hESC is most likely contributed by alterations in the metabolism of centrosomes.

Ibrahim A. Ibrahim, M.D., M.P.H., Ph.D., is the Director of Urology Outcomes Research at the William Beaumont Hospital, Royal Oak, Michigan. Dr. Ibrahim is a health services research expert who joined the Department of Urology at William Beaumont Hospital in 2005 where he directs the urology outcomes research and participates in database design and management for outcomes studies in prostatectomy, pelvic pain, urinary incontinence, and neuromodulation for urological conditions. He has been heavily involved in the design and analysis of studies looking at prevalence of interstitial cystitis, male incontinence, measuring sexual function in interstitial cystitis patients, abuse in interstitial cystitis, pelvic floor dysfunction, pelvic surgeries in interstitial cystitis and many others. Dr. Ibrahim has authored two book chapters on diabetes disease management and polypharmacy among elderly diabetic patients in home health care. He is also on the editorial boards of the International Urology and Nephrology Journal and the Health Administration Education Journal in addition to being a reviewer for the British Journal of Urology International, and the Canadian medical Association Journal and few other peer reviewed publications. At the national level, he is a reviewer for Health Resources and Services Administration grants.

Presentation Summary: Although 20 years ago scientists started studying stem cells (SC), it was only 10 years ago that human embryonic stem cells were isolated in the lab. Since then, we gained a great deal of knowledge on essential properties of stem cells and what makes them different from specialized cell types. Stem cell research holds a promise of using these cells in cell-based therapies especially in chronic, aging, and degenerative conditions. Moreover, another direction has emerged using SC for screening new drugs and toxins and understanding birth defects. However, progress in this important and promising research has been affected by the federal restrictions on embryonic stem cell research. Progress in this research is dependent on funding. In this presentation, we follow the trend in funding for SC research from federal, state, and private sources. We also present how different countries and states approached this funding dilemma and will point out the different avenues available through federal sources, even in the presence of current day restrictions. One source that has not received enough attention is the federally funded program entitled "Production Assistance for Cellular Therapies" (PACT). Details of this program will be presented.

Omar A. Khan, M.D., is Professor of Neurology and Director of the Multiple Sclerosis Center & Image Analysis Laboratory at Wayne State University School of Medicine in Detroit, Michigan. He is also Director of the Multiple Sclerosis Clinic at the Detroit Medical Center. Dr. Khan has published over 250 peer-reviewed papers, abstracts, reviews, and book chapters. He has received numerous awards and has served as faculty for the American Academy of Neurology. Dr. Khan is an elected member of the American Neurological Association and serves on the Medical Advisory Board of the National Multiple Sclerosis Society. He serves as an ad hoc reviewer for the NMSS, NIH Study Section and the Canadian Institutes of Health Research. As principal investigator, Dr. Khan has conducted several landmark exploratory and multi-center clinical trials. His areas of interest include imaging and genetics of multiple sclerosis, and developing therapeutic approaches to treat multiple sclerosis. Dr. Khan has received research funding by the National Institutes of Health, Department of Veterans Affairs, the National Multiple Sclerosis Society, and the pharmaceutical industry.

Presentation Summary: This presentation will discuss the possible abilities of MSC to induce enhanced tissue repair and regeneration. Currently, two phase I studies are underway in relapsing multiple sclerosis examining the safety and feasibility of intravenously administered MSC. Furthermore, in-vivo methods of imaging to examine myelin injury and repair at the macromolecular level will be discussed to investigate the effect of MSC on tissue injury and repair. These studies are likely to provide the proof of concept supporting the use of MSC therapy in multiple sclerosis before undertaking larger multi-center initiatives.

Senator Carl Levin (D-Michigan). In an editorial about Carl Levin, the *Detroit News* wrote, "He has been above reproach personally and has stuck to his principles, even when they were unpopular. Principled leadership, no matter what political ideology it comes from, is sorely needed in Washington." *TIME Magazine* recently named Carl Levin one of "America 's 10 Best Senators," noting that "the Michigan Democrat has gained respect from both parties for his attention to detail and deep knowledge of policy."

Senator Carl Levin has proudly served in the United States Senate since 1978 earning a reputation for dogged investigations and common sense approaches to government spending, policies and legislation. He is currently the Chairman of the Senate Armed Services Committee, introducing legislation to protect our troops while providing realistic proposals to end the war.

As Chairman of the Permanent Subcommittee on Investigations, Levin has focused on issues that impact the wallets of most Americans, including unfair credit card practices and sky-high oil and natural gas prices. He has also lead investigations into economy damaging foreign tax havens, money laundering schemes and corporate abuses including the most in-depth examination of the Enron collapse. In Michigan, Carl Levin has placed a high priority on constituent services, offering a wide array of casework and resources. Levin and his staff are accessible and eager to assist Michigan residents, earning him a reputation as one of the most responsive Senators in the nation.

Presentation Summary: Senator Levin, along with other Michigan elected officials, serves on the advisory council for the Michigan Citizens for Stem Cell Research and Cure (MCSCRC), a non-profit organization established in 2006 for the purpose of educating Michigan citizens on the potential promises of embryonic stem cell research. The non-profit works with the organization that is currently pushing for a state ballot initiative which would remove the ban on embryonic stem cell research in Michigan. Senator Levin will be discussing the potential that embryonic stem cell research holds for families and individuals battling disease as well as the political implications and struggles that lie ahead.

Tenneille Ludwig, Ph.D., obtained a B.S. (1992) in Animal Sciences and M.S. (1994) in Reproductive Endocrinology from Washington State University prior to completing a Ph.D. in Embryology and Developmental Biology with a minor in Bioethics from the University of Wisconsin-Madison in 2001. Her subsequent work in the laboratory of Dr. James Thomson (2001-2007) focused primarily on the optimization of cell culture conditions, and resulted in the development of a defined, feeder-independent culture system for human embryonic stem cells (TeSR). Dr. Ludwig is currently a senior scientist at WiCell Research Institute and leads the media optimization, distribution and quality control programs for the National Stem Cell Bank. Her primary research interest

continues to be focused on improving human ES cells culture. Specifically, current projects include investigations into improving attachment and cloning efficiency, enabling large-scale culture of human ES cell, and developing alternate high throughput screening methods.

Presentation Summary: Traditional culture systems rely on mouse embryonic fibroblast (MEF) feeder layers, which can be labor intensive to prepare, highly variable, a potential source of contamination, and limit scientific productivity in the laboratory. Here we describe the defined, feeder-independent culture systems TeSR1 and mTeSR1. Human ES cells maintained long-term in mTeSR1 remain undifferentiated, karyotypically normal, and demonstrate the potential to differentiate into a variety of lineages through embryoid body formation. To date, approximately 35 independent hES cell lines from around the globe and 8 iPS cell lines have been successfully cultured in mTeSR1.

Domenico Luongo, M.S., C.H.M.M., began his career as a research assistant in Wayne State University's Department of Internal Medicine. After seven years at the lab bench, he switched gears to the compliance side of research, utilizing his Master of Science Degree in Occupational and Environmental Health Sciences from Wayne State University's School of Pharmacy and Allied Health. Domenico is currently the Laboratory Compliance Manager for Oakland University and a senior level Certified Hazardous Materials Manager. He has been a Biosafety Officer for over eight years, with additional responsibilities as Oakland University's Radiation Safety Officer and Chemical Hygiene Officer.

Presentation Summary: Stem Cell Research is governed by a myriad of regulations and guidelines that can affect a researcher's ability to be funded and approved for their research. This workshop will provide participants with a background on the institutional responsibilities associated with stem cell research and a basic understanding of the regulations and guidelines required for keeping their research in compliance.

Ivan Maillard, M.D., Ph.D., is a physician-scientist with an interest in hematopoietic stem cells, Notch signaling and T cell immunology. He obtained his MD and PhD degrees from the University of Lausanne, Switzerland, before joining the Hematology-Oncology fellowship program of the University of Pennsylvania, Philadelphia, PA. There, in addition to working as attending physician in the leukemia and bone marrow transplantation team, he completed post-doctoral training with Warren S. Pear, MD PhD, working on the role of Notch signaling in hematopoiesis and T cell development. Dr. Maillard is currently an Assistant Professor at the University of Michigan Medical School, as well as a member of the University of Michigan Center for Stem Cell Biology.

Presenation Summary: Gain-of-function experiments have demonstrated the potential of Notch signals to expand primitive hematopoietic progenitors, but whether Notch physiologically regulates hematopoietic stem cell (HSC) homeostasis in vivo is unclear. To answer this question, we evaluated the effect of global deficiencies of canonical Notch signaling in rigorous HSC assays. Notch-deprived HSCs achieved stable long-term reconstitution of irradiated hosts, showed a normal frequency of progenitor fractions enriched for long-term HSCs and provided normal long-term reconstitution after secondary competitive transplantation. Furthermore, Notch signaling intensity appears to be actively maintained at very low levels in primitive hematopoietic progenitors. Taken together, these results rule out an essential physiological role for cell-autonomous canonical Notch signals in HSC maintenance.

Representative Andy Meisner (D-Ferndale, Michigan). Elected in 2002, Andy Meisner began his political career as a Legislative Assistant for U.S. Congressman Sandy Levin. He was responsible for policy relating to crime, community-oriented policing, community coalitions, drug courts, unemployment compensation, and foreign affairs including the Middle East. Andy also is co-founder of the Michigan Democratic Action Network, a grassroots organization with members in Michigan and Washington, D.C., that works to engage young adults in the political process.Andy received accolades from the Michigan Parent, Teacher, Student Association (PTSA) for "demonstrating outstanding leadership and advocacy for support of education on behalf of children and youth." He also received an Environmental Leadership Award by the League of Conservation Voters for having a 100% rating on their 2003 Michigan Legislative Scorecard. In addition, he has introduced bills to remove restrictions on embryonic stem cell research.

Presentation Summary: This presentation will focus on the background of the State and Federal public policy in relation to Stem Cell research. In addition, the presentation will also give an update on current Michigan efforts on lifting the ban.

Jack Mosher, Ph.D., is an Assistant Research Scientist at the University of Michigan Center for Stem Cell Biology. He received his Bachelors of Science degree from Allegheny College and a Masters of Arts from the University of Hartford. After spending two years working in a drug discovery laboratory at a pharmaceutical company, he obtained his Ph.D. from the University of North

Carolina at Chapel Hill where he studied the transcriptional control of early nervous system development. He joined the laboratory of Dr. Sean Morrison in the Life Sciences Institute at the University of Michigan as a Postdoctoral Fellow in 2001. His postdoctoral research focused on understanding how adult stem cells contribute to the development and disease of the peripheral nervous system. Currently he is trying to apply the knowledge gained from animal studies to develop models of human peripheral nervous system using human embryonic stem cells.

Presentation Summary: During embryonic development neural crest stem cells migrate the length of the embryo to generate the enteric nervous system (ENS), which regulates gastrointestinal function. Defects in this migration can lead to the incomplete formation of the ENS that may manifest clinically as Hirschsprung disease. My presentation will define neural crest stem cells and their role in development of the ENS, describe how defects in their migration lead to Hirschsprung disease and how this defect can be overcome by the transplantation of neural crest stem cells.

Andras Nagy, Ph.D., is currently a Senior Scientist at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital and a Professor in the Department of Molecular Genetics at the University of Toronto. He also holds a Tier I Canada Research Chair in Stem Cells and Regeneration. His research focuses on several areas of interest, which include 1) Functional studies of genes belonging to families with known roles in vessel formation; 2) Development of sophisticated genetic manipulation tools in the mouse model; 3) Applying genetics to cancer research; 4) Derivation, differentiation and genetic modification of both mouse and human Embryonic Stem cells; 5) Reprogramming of somatic cells to pluripotent stem cells. Dr. Nagy¹s research is currently funded by the National Cancer Institute of Canada, Genome Canada, Stem Cell Network, JDRF, and the National Institutes of Health USA.

Presentation Summary: In 2007, The Nobel Prize in Physiology or Medicine was awarded to the pioneers of mouse Embryonic Stem cells-based genetics, which revolutionized and accelerated mouse genetics at an unprecedented level. Throughout this talk, details will be given on the ongoing international efforts aiming to generate mutations in all mouse genes during the next four years. The Canadian effort (NorCOMM) utilizes unique state-of-art genetic instruments to achieve targeted mutations, which are easily replaceable with any desired vector from a toolbox. These new developments in stem cell based genetics are rapidly changing the landscape and will have an impact on the way we utilize genetics for basic and medical research.

Jack Parent, M.D., received his undergraduate degree at Stanford University and his M.D. at Yale University School of Medicine. He completed medical internship, neurology residency, clinical fellowship, and post-doctoral research fellowship training at the University of California, San Francisco. Dr. Parent established his laboratory at the University of Michigan in 2000, where he studies neural stem cell biology and the effects of brain injury on adult neurogenesis using animal models of epilepsy and stroke. He has received several awards for his research, including a Junior Investigator Award from the American Epilepsy Society, a Paul Beeson Physician Faculty Scholars in Aging Award, the Dreifuss-Penry Epilepsy Award from the American Academy of Neurology and the Grass Foundation Award in Neuroscience from the American Neurological Association . Dr. Parent's current research interests include mechanisms of postnatal brain development and neural stem cell regulation, the influence of brain injury on adult neurogenesis, and the use of neural stem cells for reparative therapy of brain disorders.

Presentation Summary: This presentation will cover current findings that promoter- or surface marker-based selection methods yield enriched NP populations that will be useful for optimizing transplantation therapy.

Graham Parker Ph.D., is a member of the division of Neurology at the Children's Hospital of Michigan in the department of Pediatrics, Wayne State University School of Medicine. His current research collaboration centers on development of in vitro and in vivo models of neurological conditions, specifically, spinal muscular atrophy. He has a long standing interest in the effects of commonly used and abused substances such as coffee, alcohol and nicotine on somatic stem cell populations. Graham is also Editor-in-Chief of an international peer-reviewed multi-disciplinary journal, Stem Cells and Development.

Presentation Summary: Stem cell research and its communication have become, for better and worse, a matter of great public interest. The peer review process is still alive and well, but has taken a severe beating of late. This presentation will touch on some delicate issues relevant to the process, such as motivation behind paper submission, reviewer recommendation and selection. Stem cell researchers are a truly an international community, although communication is primarily in English. However, in spite of international recognition of the importance of ethics in the research and publication process, cultural and regulatory differences create a gulf of perspective that warrants consideration.

Bruno Péault, Ph.D., is recognized for his research on the identification, characterization and purification of several categories of human stem cells, is a Professor in the Departments of Pediatrics, Orthopaedics and Cell Biology at the University of Pittsburgh, as well as Co-Director of the Stem Cell Research Center at Children's Hospital of Pittsburgh of UPMC. Prior to joining the McGowan

Institute in 2003, he served as the Research Director, Centre National de la Recherche Scientifique (CNRS), and department head at the Institut National de la Santé et de la Recherche Médicale (INSERM). Dr. Péault's stem cell based research has gained him notoriety for his work with hematopoietic stem cells, mesenchymal stem cells and endothelial stem cells, as well as pancreas and respiratory epithelium stem cells.

Presentation Summary: I will describe the prospective identification and purification of multi-lineage progenitor cells in human organs.

Jose L. Garcia-Perez, Ph.D., has been working in the field of mobile DNA for the past 10 years. He obtained his Ph D. from the University of Granada/Spanish Research Council (CSIC), Spain in 2003. His Ph D. work analyzed the enzymatic machinery encoded by a non-LTR retrotransposon from *Trypanosoma cruzi* (L1Tc). In 2003, he joined the laboratory of Dr. John V. Moran at the University of Michigan Medical School, in the Department of Human Genetics. The focus of the Moran lab is the impact of LINE-1 retrotransposons in the human genome. During the past 5 years, Dr. Garcia-Perez's work within the group led by Dr. Moran has facilitated recapitulation of LINE-1 retrotransposition in human embryonic stem cells, which suggest that new LINE-1 insertions can be accumulated during early stages of human development. He is currently a Faculty Member in the Department of Human Genetics (Research Investigator) and his main focus is the impact of LINE-1 retrotransposons in several human stem cell types.

Presentation Summary: The vast majority of the human genome is made up of repeated DNA, which encompasses several classes of retroelements. LINE-1 (or L1) retrotransposons constitute 17% of human DNA, and there are estimated to be 80-100 retrotransposition-competent L1 elements in the genome of each human being. However, little is known about how and when new L1 insertions arise during the development of an individual. In order to understand how *de novo* insertions are generated, it is important to determine what cell type(s) are able to accommodate L1 retrotransposition. Human embryonic stem cells (hESCs) represent an excellent model to recapitulate LINE-1 retrotransposition in early stages of development. We recently have demonstrated that endogenous LINE-1 retroelements are expressed in hESCs, and that engineered L1 elements are able to undergo retrotransposition in hESCs. Thus, our data indicate that L1 may be able to retrotranspose early in development, giving rise to *de novo* insertions in the genomes of newborn individuals.

Pamela Gehron Robey, Ph.D., is currently Chief of the Craniofacial and Skeletal Diseases Branch, NIDCR, NIH. Her work has focused on the isolation and characterization of osteogenic cells and factors that mediate their differentiation and production of bone extracellular matrix. She developed a technique for establishment of normal, non-transformed osteogenic cells in vitro, a method that is now broadly used, which enabled the isolation and characterization of many of the genes for osteogenic proteins. She subsequently turned towards the identification of osteogenic precursor cells in bone marrow, bone marrow stromal ("mesenchymal") stem cells, and has been studying their multi-potential nature, their role in skeletal diseases, and their use in regenerative medicine. Dr. Robey has served on numerous editorial boards, is a past Associate Editor of the Journal of Bone and Mineral Research, and currently is a Lead Reviewer for Stem Cells. She serves on many committees in professional organizations, in particular, on those focused on the career development of young investigators. She is a member of the NIH Stem Cell task force, and as such, she is often called upon to give presentations regarding stem cell biology to scientific and lay audiences.

Presentation Summary: Skeletal stem cells are a subset of bone marrow stromal cells that are clonogenic, and able to form bone, cartilage, myelosupportive stroma and marrow adipocytes. In addition to participating directly in bone formation, they also control bone resorption by virtue of the fact that they support and control the formation of osteoclasts, thereby making these cells central mediators of post-natal skeletal homeostasis. As such, any genetic change, or change in the microenvironment in which they reside that alters their biological behavior will result in a skeletal disease or disorder, thereby making them a potential target for therapy. In addition, by virtue of their extensive proliferative ability, ex vivo expansion of bone marrow stromal cells while maintaining the skeletal stem cell provides a promising cell source for reconstruction of bone lost due to trauma or disease.

Peter Sartipy, Ph.D., is a Senior Scientist and Project Manager at Cellartis AB. He received his M.Sc. in Chemical Engineering in 1994 from Chalmers University of Technology (Göteborg, Sweden). He then went on to earn his Ph.D. in 2000 from the Faculty of Medicine at Göteborg University. After working as a post-doc at the Department of Cell Biology at The Scripps Research Institute (La Jolla, CA, USA) he returned to Göteborg and joined Cellartis AB in 2002. His current research is mainly directed at exploring human embryonic stem cell differentiation towards cardiomyocytes and development of novel drug discovery applications based on these cells.

Presentation Summary: Improved technologies are urgently needed in order to develop more effective and safe new drugs and for reducing late stage attrition. The possibility to propagate undifferentiated hES cells and subsequently differentiate them into desired target cell types will provide a stable supply of cells for a range of applications. Functional cardiomyocytes and hepatocytes can be derived from hES cells and the differentiated cells share similarities with their adult counterparts. These results lend support to the further development of assay systems based on these cells and novel opportunities and challenges of using hES cells and derivatives thereof in drug discovery will be discussed.

Jill Slater, B.S., is a physiology graduate research assistant in the School of Medicine at Wayne State University. Her current research involves how the stress enzyme activation tips the balance from pluripotency to differentiation in embryonic and trophoblast stem cells.

> *Presenation Summary:* We have found that cellular stressors downregulate the expression of transcription factors that maintain potency in ES and TS cells. A stress-response mechanism shared by embryonic and trophoblast stem cells activate those enzymes which induce the downregulation of pluripotency-maintaining transcription factors and through the release of inhibition, lead to the upregulation of differentiation-mediating factors.

Dave Svinarich, Ph.D., is currently the Director of Research for Providence Hospital in Southfield, Michigan and serves as the Cardiovascular Research Network Director for St. John Health. He is also an adjunct professor in the departments of Obstetrics and Gynecology, Immunology and Microbiology and the Center for Molecular Medicine and Genetics at the Wayne State University School of Medicine. Dr. Svinarich received his undergraduate degree from the University of Michigan and his Doctoral degree from Wayne State University, where he also served as an Associate Professor in the department of Obstetrics and Gynecology. He serves on or directs biosafety committees, Institutional Review Boards and Animal Investigation Committees. He also serves as a research consultant for local hospitals.

> *Presentation Summary:* The presentation will discuss regulatory issues governing hES research in the United States. In specific, the role and limitations of the Institutional Review Board, FDA, Animal Investigation Committee and the Embryonic Stem Cell Research Oversight (ESCRO) Committee, will be addressed.

Shuichi Takayama, Ph.D., is associate professor in the Department of Biomedical Engineering and the Macromolecular Science and Engineering Program at the University of Michigan, Ann Arbor. He received his M.S. from the University of Tokyo in 1994 and his Ph.D. degree in chemistry from the Scripps Research Institute in 1998, after which he did postdoctoral studies at Harvard University as a Leukemia and Lymphoma Society postdoctoral fellow. He joined the faculty of the department of Biomedical Engineering at the University of Michigan, Ann Arbor, in the fall of 2000. His major research interests are in biomaterials, nanotechnology, microfluidics, and application of these technologies for cell-based therapies and biological studies.

> *Presentation Summary*: Many biological studies, drug screening methods, and cellular therapies require culture and manipulation of small numbers of precious living cells outside of the body. The gap between the cellular microenvironment in vivo and in vitro, however, poses challenges for obtaining physiologically relevant responses from cells used in basic biological studies or drug screens and for drawing out the maximum functional potential from cells used therapeutically. One of the reasons for this gap is because the fluidic environment of mammalian cells in vivo is 3D, microscale, and dynamic whereas typical in vitro cultures are 2D, macroscopic, and static. This presentation will give an overview of efforts in our laboratory to develop programmable microfluidic systems that enable spatio-temporal control of both the chemical and fluid mechanical environment of cells. The technologies and methods close the physiology gap to provide biological information otherwise unobtainable and to enhance cellular performance in therapeutic applications. Specific biomedical topics that will be discussed include in embryo culture on a chip and microfluidic formation and culture of embryoid bodies.

Dean Tantin, Ph.D., is a Assistant Professor. B.S., *magna cum laude*, Molecular Biology, University of California, San Diego, 1988-1992. Ph.D., Molecular Biology Institute Interdepartmental PhD Program, University of California, Los Angeles, Advisor: Dr. Michael Carey, 1992-1997. Postdoctoral Fellow, Gene Regulation & Molecular Genetics, Laboratory of Phil Sharp, Massachusetts Institute of Technology, Center for Cancer Research, 1998-2005. Assistant Professor, University of Utah School of Medicine, Department of Pathology, Division of Cell Biology & Immunology, 2006-present. Funding sources: American Cancer Society Research Scholar Grant, March of Dimes/Basil O'Connor Starter Scholar Award.

> *Presentation Summary:* The Oct4 transcription factor is a master regulator of "stemness"--a phenomenological term generally lacking molecular correlates. Oct1 is a homologous protein expressed in adult cells. We show: 1) "stemness" is, at least in part, a metabolic phenomenon related to the Warburg effect that is controlled by Oct1 and Oct4. 2) Oct1 and Oct4 regulate cellular sensitivity to stress agents. 3) Oct1 and Oct4 have similar mechanisms of upstream regulation. 4) Oct1 and Oct4 use similar mechanisms of downstream transcriptional regulation. 5) Oct1 is a potent regulator of carcinogenesis.

Barbara M. Tigges, Ph.D., graduated from the University of Wisconsin-Oshkosh with a Bachelor of Science degree in Microbiology and Public Health. She went on to further her studies and completed a Ph.D. degree program at Finch University of Health Sciences/The Chicago Medical School in Microbiology and Immunology. Her specialty was in the field of Infectious Disease primarily focused on HIV-1 mediated immune responses. She obtained a Scientist position at BioE, Inc. (St. Paul, Minnesota) in 1999 initially in the development of immunoassays pertaining to a broad spectrum of infectious diseases. Other key aspects of involvement were related to cytokines, cellular bioassays, and cell separation reagents. In 2002, Dr. Tigges was promoted to a Senior Scientist level and shortly thereafter began her work in the stem cell field with the isolation, characterization, and differentiation of umbilical cord blood-derived Multi-Lineage Progenitor Cells (MLPC). She has been a scientist at BioE, Inc. for approximately the past nine years. Dr. Tigges currently resides in the Twin Cities area.

Presentation Summary: Multi-Lineage Progenitor Cells (MLPC) are normal diploid cells derived from post-partum human umbilical cord blood which have the capacity to be extensively expanded. They have been fully characterized as a novel stem cell with a normal karyotype that is highly uncommitted toward a particular lineage and is genetically and phenotypically distinct from a mesenchymal stem cell. MLPC are capable of being differentiated into several cell types derived from and respresentative of each embryonic layer: mesoderm, ectoderm, and endoderm. Regarding regenerative medicine, stem cells possess great potential within the tissue engineering and cellular therapy fields. Their capability to differentiate into multiple lineages creates an attractive target for therapeutic purposes as well as several pharma applications.

Donggang Yao, Ph.D., Associate Professor in the School of Polymer, Textile and Fiber Engineering at Georgia Tech, received a BS degree in Precision Instruments from Shanghai Jiao Tong University in 1991 and MS and Ph.D. degrees in Mechanical Engineering from University of Massachusetts Amherst in 1998 and 2001, respectively. He joined Georgia Tech in 2004. He teaches and directs research in the broad area of polymer engineering. His ongoing research deals with polymer microfabrication, single-polymer composites manufacturing, and productionof novel polymer porous structures. The research in these areas has resulted in about 40 journalpublications, 50 conference proceedings papers, and 60 technical presentations. He was a recipient of the NSF Career Award in 2003 for his research on rapid-production of polymer based microstructures. He served as organizers for several symposiums in international ASME and SPE conferences.

Presentation Summary: This presentation will discuss the fabrication of functional porous structures for tissue engineering. The biocompatibility of the polycaprolactone scaffolds with a porosity of approximately 70% was verified using scaffold seeding experiments with stem cells and their derivatives. These scaffolds were found to promote the proliferation and growth of stem cells differentiated osseous cells.

Sichang Zhou, B.S., is a graduate student at the School of Medicine at Wayne State University. He currently works in Dr. Dan Rappolee's laboratory in the Department of Physiology and Reproductive Sciences: OB/GYN. His research investigates how the long-term effects of stress on embryonic and trophoblast stem cells is through global changes in effectors of differentiation.

Presentation Summary: In TSC culture, FGF4 removal emulates normal differentiation, but we have determined that addition of hyperosmolar stress to TSC causes global changes in mRNA transcription that are dominant over FGF4 and cause differentiation. In, ESC it is anticipated that the factors mediating endoderm differentiation, such as GATA4 and GATA6, will be upregulated on the protein and mRNA levels by hyperosmolar stress.

PRESENTATION ABSTRACTS

Friday May 9, 2008

Workshops 3:00 pm – 4:00 pm

Muscle Derived Stem Cell Therapy of Stress Incontinence

Michael B. Chancellor, M.D.
Department of Urology, William Beaumont Hospital, Royal Oak, Michigan

The role of a medical doctor as a physician scientist is the goal of translational research. Medical doctors doing research need to focus on basic science in the research laboratory bench top that will be translated into new treatment for our patients at the bed side. I will share with you my journey on the application of muscle stem cell therapy for stress urinary incontinence.

Approximately 13 million Americans are living with urinary incontinence -- a condition that causes the bladder to leak urine. It's nearly twice as common in women, and many don't seek help. Surgical slings, pills and exercises are a few common treatments, but researchers say stem cell therapy could offer new hope to people looking to live a life free of embarrassment.

To perform stem cell therapy, doctors take a small muscle biopsy from the patient's leg and send it to a lab where researchers isolate and grow the stem cells. A few weeks later, these muscle cells are injected into muscles around the urethra to strengthen it and prevent leakage.

The procedure itself takes just five minutes, and 60 percent of patients surveyed in the first North American study reported improvement after one year, with no side effects. The recovery time is also short. Patients can go home and be active right away, as opposed to other surgeries where they need to restrict their activity while things heal. http://www.ivanhoe.com/newsalert/.

Building on the pioneering work of Drs Michael Chancellor and Johnny Huard, and under a license agreement with the University of Pittsburgh, Cook MyoSite Inc., is developing technology involving the use of autologous muscle-derived cells for treating urinary incontinence and other diseases. These autologous cells - cells taken from the patient - may also have the ability to repair other damaged tissues.

Cook MyoSite co-founders, Michael Chancellor, M.D., and Johnny Huard, Ph.D., partnered in 1997 to develop muscle stem cell based therapies. In 2001, with funding from Indiana-based Cook Medical, they founded Cook MyoSite. The company has since then completed the first North American trial using muscle-derived cells to treat urinary incontinence and has started a multi-center trial in Canada. Cook MyoSite's new headquarters is located at the Regional Industrial Development Corporation (RIDC) Park and outfitted with state of the art U.S. Food and Drug Administration (FDA) Good Manufacturing Practice (GMP) cellular manufacturing equipment.

Funding for Stem cell Research, Past, Present, and Future

Ibrahim A. Ibrahim, MD, MPH, PhD
William Beaumont Hospital, Royal Oak, Michigan

Although 20 years ago scientists started studying stem cells (SC), it was only 10 years ago that human embryonic stem cells were isolated in the lab. Since then, we gained a great deal of knowledge on essential properties of stem cells and what makes them different from specialized cell types. Stem cell research holds a promise of using these cells in cell-based therapies especially in chronic, aging, and degenerative conditions. Moreover, another direction has emerged using SC for screening new drugs and toxins and understanding birth defects. However, progress in this important and promising research has been affected by the federal restrictions on embryonic stem cell research. Progress in this research is dependent on funding. In this presentation, we follow the trend in funding for SC research from federal, state, and private sources. We also present how different countries and states approached this funding dilemma and will point out the different avenues available through federal sources, even in the presence of current day restrictions. One source that has not received enough attention is the federally funded program entitled "Production Assistance for Cellular Therapies" (PACT). Details of this program will be presented.

Opening Session 6:00 pm – 8:00 pm

An Ubiquitous Reserve of Perivascular Multi-lineage Stem Cells within Human Tissues

Bruno Péault, Ph.D.
University of Pittsburgh, PA

Multi-lineage stem cells were believed to be present exclusively in developing embryos, and from these early stages be lost progressively at the expense of committed tissue progenitors. This idea was challenged by the discovery that multipotent stem cells can be derived from diverse adult organs such as the brain, bone marrow, skeletal muscle or even fat tissue. However, adult multipotent stem cells were exclusively derived from long-term cultured cell suspensions. The identity, anatomic localization and even existence within native adult tissues of such multi-lineage stem cells therefore remained obscure. We have attempted to prospectively identify, purify and characterize such broadly committed progenitors within fetal and adult human human tissues that include placenta, pancreas, skeletal muscle, lung, bone marrow and adipose tissue. We now present evidence for the presence in all human tissues of multi-lineage progenitor cells associated with the walls of blood vessels.

First, we have documented anatomic, molecular and developmental relationships between endothelial and myogenic cells within human skeletal muscle. Cells co-expressing myogenic and endothelial cell markers (CD56, CD34, CD144) have been identified by immunohistology and flow cytometry. These *myo-endothelial* cells form myotubes *in vitro* and regenerate myofibers in the SCID mouse muscle about ten times more efficiently than CD56+ myogenic progenitors. Cultured CD56+CD34+CD144+ cells proliferate long-term, retain a normal caryotype, are not tumorigenic and better survive under oxidative stress than CD56+ myogenic cells. Clonally derived CD56+CD34+CD144+ cells were also found to differentiate into bone and cartilage cells in culture.

Next, we have identified, by immunohistochemistry and flow cytometry, perivascular cells know as pericytes in a variety of pre- and postnatal human tissues including skeletal muscle, pancreas, bone marrow, brain, adipose tissue, skin and placenta, according to CD146, NG2, CD133 and PDGF receptor expression and absence of CD56, CD45, CD34 and other endothelial cell markers. Perivascular cells purified to homogeneity by flow cytometry from skeletal muscle as well as non-muscle tissues were robustly myogenic in culture and when transplanted into the cardiotoxin-injured skeletal muscles of immunodeficient mice. Purified human perivascular cells were established in long-term culture where they retained their markers and myogenic developmental ability but, also, exhibited osteogenic, chondrogenic and adipogenic potentials. Strikingly, long-term cultured human perivascular cells expressed all the known markers of mesenchymal stem cells (MSC) such as CD44, CD73, CD90 and CD105. These data suggest the existence of perivascular cells at the origin of the elusive mesenchymal stem cells, which have been so far identiifed only retrospectively in primary cultures of whole organs.

Finally, we show that following transplantation into the infarcted hearts of SCID mice both human myoendothelial cells and pericytes can significantly improve cardiac anatomy, as measured by scar area and ventricle diameter reduction, and contractility, assessed by percent fractional shortening and percent fractional area change.

Altogether, these results confirm the key role that blood vessel walls appear to play as progenitor cell providers in development and post-natal life. These novel human blood-vessel associated progenitor cells are amenable to biotechnological handling, including purification by flow cytometry and clonal multiplication *in vitro*, and may therefore represent convenient therapeutic cells for human tissue repair.

How Do hESC Sense and Respond to Damage to their DNA?

Ales Hampl, D.V.M , Ph.D.,[1,2], Zuzana Holubcova[2], Tomas Barta[1,3], Sarka Pospisilova[4], Jan Verner[4], Dasa Dolezalová[1,3], Vladimir Vinarsky[2], Petr Dvorak[1,2]

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[4] Center of Molecular Biology and Gene Therapy, Department of Internal Medicine-Hematooncology, University Hospital and Faculty of Medicine, Masaryk University, Czech Republic

Human embryonic stem cells (hESC) are immature cells derived from human blastocyst-stage embryos that can be indefinitely propagated in culture and can also be induced to differentiate into all mature cell types with special function. These two abilities make hESC attractive tool for cell replacement therapy, drug development, and toxicology. Importantly, any risk of unpredictable behavior of hESC must be eliminated before these cells are used for therapy. Several studies have recently reported accumulation of various types of alterations to DNA of hESC that could result in deregulated growth of hESC derivatives upon their transplantation

to the patient. Which types of stress induce such damage, contribution of hESC genotype, what are the biological outcomes of such damage, and many other questions remain to be answered. As numerical and structural abnormalities of centrosomes contribute to chromosomal instability in many cancers, we have assessed the metabolism of centrosomes in hESC. We have found in several independent cells lines that undifferentiated hESC are typical by unusually high frequency of mitoses with the number of centrosomes exceeding two, which participate in formation of multipolar spindles. Although centrosome amplifications occur in high frequency (10-30%) in hESC in early to mid passages they become suppressed in hESC in later passages (less then 5%). We have found that at least two of them, Aurora A and CDK2, are overabundant and highly active in undifferentiated hESC and may thus contribute to the observed abnormalities. This is supported by the fact that chemical inhibition of CDK2 in hESC reduces the percentage of mitoses with supernumerary centrosomes. Both the integrity and number of centrosomes seem to be monitored by signaling pathways that employ established molecules involved in sensing and executing response to DNA damage. Here we show that at least some components of such pathways are developed and operative in hESC. Upon DNA damage induced by UVC-irradiation damage, hESC accumulate p53 protein that is capable of transactivation of its target genes. UVC-irradiated hESC also phosphorylate Chk2, degrade their CDC25A phosphatase, and, according to at what stage of cell cycle hESC are exposed to DNA damaging insult, delay their cell cycle in G1 or G2/M phase.
In summary, we demonstrate that although undifferentiated hESC possess some protective mechanisms to maintain its DNA pristine, they still develop conditions that are favorable to generation of genetic abnormalities. We propose that understanding of mechanisms involved in harboring early alterations to genome of originally genetically normal hESC, and their link to the changes in their phenotype in particular, will shed some light on initial steps of carcinogenesis.

Remodeling Injured Brain with Cell Therapy

Michael Chopp Ph.D.
Oakland University, Rochester, MI; Henry Ford Hospital, Detroit, MI

Key words: *cell-based therapy, neurological disease, stroke, bone marrow mesenchymal cells*

The primary action of cell-based therapy in inducing recovery of function post brain injury is to stimulate endogenous restorative mechanisms and not to replace tissue. The cells, whether they be bone marrow mesenchymal (MSC), neurospheres, umbilical cord blood or fetal or embryonic cells, when injected into the adult do not repopulate the adult brain tissue; they produce an array of factors including angiogenic and neurotrophic factors which initiate the restorative cascade of recovery.

In this presentation, I will describe our data on the multiple therapeutic targets of cell-based treatment of neurological disease, including stroke, (ischemic, hemorrhagic), traumatic brain injury and multiple sclerosis. Stroke will be the primary model to illustrate efficacy and to elucidate mechanisms that underlie therapeutic benefit.

In our studies of stroke, we induce middle cerebral artery occlusion (MCAo) by the placement of a monofilament into the internal carotid artery to transiently (2h) or permanently block the MCA. This produces a massive unilateral infarction which encompasses nearly the entire territory of the MCA and results in major and persistent neurological deficits. We have treated this type of lesion with a number of different cell types; however, we will focus the present data on treatment with bone marrow mesenchymal cells. All the animals are subjected to a battery of outcome measures which include a modified neurological score, adhesive removal tests, among others. Our data show that there is significant and persistent therapeutic benefit when MSCs are administered via a vascular route 1, 7 or 30 days after stroke onset. Some of the treated animals have been permitted to survive for one year and at which time significant functional benefit persists. We have also performed extensive investigations of therapeutic window and dose-response for treatment of neural injury and neurodegenerative disease.

Mechanisms of action have been extensively studied in our laboratory. Essentially, we find that MSCs stimulate a tapestry of restorative events in the brain and spinal cord. MSCs catalyze the expression of a variety of trophic factors, chemokines and angiogenic proteins by parenchymal cells which weave restorative processes. These restorative events include enhanced neurogenesis, angiogenesis , and synaptogenesis, in addition to reductions in glial scar formation and inhibitory glycoproteins. Treatment of stroke, for example, induces rewiring of neurites in the spinal cord and brain, along with other beneficial events. Many of these events, including angiogenesis and white matter restructuring can be non-invasively monitored using MRI.

The overwhelming evidence from many preclinical studies is that MSC therapy is primed for treatment of stroke, trauma, MS and possibly other neurological diseases and pathological states.

Saturday May 10, 2008

Stem Cell Biology Session I 8:15 am – 10:00 am

Long Term Stability and Differentiation Potential of Human Embryonic Stem Cells Cultured in Defined Medium

Tenneille Ludwig, Ph.D.
WiCell Research Institute and the National Stem Cell Bank

Human embryonic stem (ES) cells offer tremendous potential for use in research and regenerative medicine, but for ES cells to be used clinically, safety will become of paramount importance. Traditional culture systems rely on mouse embryonic fibroblast (MEF) feeder layers, which can be problematic. In addition to being a source of potential contamination, MEFs are a major source of variability in culture and are labor intensive to prepare. Derivation and routine production of MEFs sufficient to support an active laboratory can drain financial and technical resources. Moving to a feeder-independent culture system allows the reallocation of those resources from MEF production to scientific discovery. We previously reported the development of TeSR1, a serum-free, animal protein-free medium specifically formulated for the feeder-independent proliferation of human ES cells *in vitro*. Although the derivation of new human embryonic stem cell lines in those defined conditions offered an important proof of principle, the costs of some of the defined components in that culture system make it impractical for everyday research use. Subsequent modifications to the media (mTeSR1) include the use of animal sourced proteins (BSA, Matrigel) and cloned zebrafish bFGF. These modifications significantly reduced the cost while still allowing feeder-independent culture, making it a practical alternative to the previous formulation (TeSR) for the common research laboratory. mTeSR1 promotes long-term proliferation of undifferentiated human ES cells in the complete absence of feeder layers. Human ES cells cultured in mTeSR1 for more than 40 passages maintain a normal karyotype, and upon FACS analysis express appropriate markers for pluripotency (>90% Oct4, SSEA4, Tra 1-60, Tra 1-81). Furthermore, human ES cells cultured in mTeSR1 for more than 20 passages readily form well organized embryoid bodies. RTPCR analysis of total RNA collected from embryoid bodies demonstrates the presence of ectoderm (NeuroD1, NF-H), endoderm (HNF3□, AFP) and mesoderm (Brachyury, cAcT) markers. Immunostaining of disassociated embryoid bodies further confirms the presence of all three germ layers: ectoderm (□ III tubulin), ectoderm (□-feto protein), and mesoderm (□-actin). Therefore, human ES cells maintained long-term in mTeSR1 remain undifferentiated, karyotypically normal, and demonstrate the potential to differentiate into a variety of lineages through embryoid body formation. To date, approximately 40 independent hES cell lines and 8 iPS cell lines have been successfully cultured in mTeSR1.

Renal Epithelial Stem Cells and Development

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During development, pluripotent embryonic stem cells become increasingly restricted in their eventual fates as cell lineage decision are made in a sequential manner. Cells of the mammalian epiblast are still pluripotent until they migrate through the primitive streak during gastrulation to generate ectoderm, mesoderm, and endoderm. Subsequently, mesodermal cells become compartmentalized into axial, intermediate, and lateral plate mesoderm, all of which contribute to different tissues. Unique combinations of transcription factors and cell signaling proteins mark the intermediate mesoderm, which is fated to make the renal and urogenital systems. The DNA binding protein Pax2 is the earliest marker for the intermediate mesoderm. Deletion of Pax2 in the germ line completely blocks renal development *in vivo*. Moreover, activation of Pax2 in ES cells, using a combination of mesoderm inducing factors, can generate renal stem cells that are able to contribute to developing kidneys. How does expression of Pax2 and other DNA binding proteins determine the developmental fate of a particular region or stem cell? To address this question we have undertaken a variety of genetic and biochemical lines of experimentation. In a model system, Pax2 promotes assembly of a histone H3K4 methyltransferase complex through interactions with the ubiquitously expressed nuclear factor PTIP (Pax Transcription activation domain Interacting Protein). We show that PTIP co-purifies with the MLL family proteins ALR and MLL3 and interacts with many components of a histone methyltransferase complex including WDR5, Ash2L, and Rbbp5. The PTIP localizes to a Pax2 DNA binding sequence, in a Pax2 dependent manner, and promotes assembly of the ALR complex to methylate H3K4. In the absence of PTIP, the Pax2 protein binds to its recognition sequence but fails to promote assembly of the ALR complex. *PTIP* germline null mutants show reduced levels of methylated histone H3K4 in neural and mesoderm derived tissues but not in the extraembryonic tissues. A reduction in global histone H3K4 methylation is also observed in a conditional *PTIP* allele in the developing neuroepithelium. These data demonstrate that PTIP is a novel component of a histone methyltransferase complex that links DNA binding proteins to epigenetic imprinting and suggest that Pax2 and related proteins determine cell lineages through epigenetic modifications of chromatin.

While Pax2 is essential for specifying the fate of intermediate mesoderm, other proteins function to renew the renal stem cell population during kidney growth. One such example is the Six2 gene product, which appears to inhibit differentiation and allow for continued proliferation of the Pax2 positive mesenchyme. Loss of Six2 promotes precocious differentiation without stem cell renewal, resulting in a rudimentary non-functional kidney. How the intrinsic growth, differentiation, and cell lineage specific signals are integrated during kidney patterning and growth will be discussed.

From Bronchial Myogenesis to the TIP Family of Transcription Regulators

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Keywords: *mesenchymal cell precursors, mechanical tension, tension induced/inhibited proteins, SANT domain, p300*

Bronchial smooth muscle (SM) develops from local mesenchymal cell precursors and extends in a proximal to distal manner. Mechanical tension, either generated by cell spreading/elongation or stretch plays an important role in initiating bronchial myogenesis. While studying the effects of mechanical tension we identified TIP-1 and TIP-3 (Tension Induced/inhibited Protein-1 and 3) in mouse lung embryonic mesenchymal cells. TIP-1 was induced on stretching, a critical signal for smooth muscle differentiation, it associated with the promoter of SRF, a key myogenic transcription factor, and induced myogenesis. On the contrary, TIP-3 was suppressed by stretch. In non-stretched lung embryonic mesenchymal cells TIP-3 was recruited to the promoter of PPARγ2 a key adipogenic transcription factor and induced adipogenesis. Here we report that the TIP family comprises eight isoforms, all bearing a SANT (for "switching-defective protein 3 [Swi3], adaptor 2 [Ada2], nuclear receptor co-repressor [N-CoR] and transcription factor [TF] IIIB) domain and some of them presenting SAM and nuclear receptor binding (NRB) motifs, all characteristic of histone modifying enzymatic complexes. TIPs have SANT-dependent, p300-mediated histone acetyltransferase (HAT) activity. Furthermore we reveal the involvement of TIP-6, the only TIP with a SANT(+)/SAM(-)/NRB(-) make up, in the induction of PPAR□2-mediated adipogenesis.

To Be or Notch to Be: What Notch Does and Does Not Do In the Hematopoietic System

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Notch signaling is a highly conserved signaling pathway that regulates cell fate decisions and tissue homeostasis in multiple contexts. Gain-of-function experiments have demonstrated the potential of Notch signals to expand primitive hematopoietic progenitors, but whether Notch physiologically regulates hematopoietic stem cell (HSC) homeostasis *in vivo* is unclear. To answer this question, we evaluated the effect of global deficiencies of canonical Notch signaling in rigorous HSC assays. Hematopoietic progenitors expressing dominant negative Mastermind-like1 (DNMAML), a potent inhibitor of Notch-mediated transcriptional activation, achieved stable long-term reconstitution of irradiated hosts and showed a normal frequency of progenitor fractions enriched for long-term HSCs. Similar results were observed with cells lacking CSL/RBPJ, a DNA-binding factor that is required for canonical Notch signaling. Notch-deprived progenitors provided normal long-term reconstitution after secondary competitive transplantation. Furthermore, Notch target genes were expressed at low levels in primitive hematopoietic progenitors, suggesting that HSCs experience a low intensity of Notch signaling in vivo. In contrast, Notch signals were essential for the generation of marginal zone B cells in the spleen and at the earliest stages of T cell development in the thymus. Furthermore, emerging evidence shows that Notch signals play important roles in the differentiation of mature T cells during a variety of immune responses. Taken together, these results rule out an essential physiological role for cell-autonomous canonical Notch signals in HSC maintenance. In contrast, our data indicate that Notch signals are essential regulators of multiple steps during lymphoid development and differentiation.

Stem Cell Therapy Session I 8:15 am – 10:00 am

MesenchymalStem Cell Therapy in Multiple Sclerosis

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Mesenchymal stem cells (MSC) are multipotent progenitor cells that have the capacity to differentiate into tissues of mesenchymal lineage making them as attractive strategy in regenerative medicine. More recently, MSC have been demonstrated to have unique immunologic properties expanding the potential of MSC therapy to autoimmune diseases as well as graft versus host disease.

Mesenchymal stem cells can inhibit the proliferation of T, B, and dendritic cells. Mesenchymal stem cells can also impair dendritic cell maturation, and therefore, antigen presentation. This has led to the interest of investigating MSC therapy in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Intravenously administered MSC in mice immunized with myelin oligodendrocyte glycoprotein significantly improved the clinical severity of EAE. In the adoptive transfer model of EAE, T-cells activated in the presence of MSC induced a milder disease compared to control T cells. The MSC-treated cells showed decreased production of IFN-γ and TNF-α. Furthermore, these cells demonstrated anergy by failing to proliferate on antigen recall. Additionally, EAE studies have shown the presence of intravenously administered green fluorescent protein-transfected MSC in the brain confirming their ability to reach the brain in an inflammatory CNS disease model. It appears that MSC effectively ameliorate EAE through a variety of mechanisms including inhibition of proliferating B and T-cell proliferative responses, antigen presentation, induction of anergy, and downregulation of Th2 cytokine production. The ability of MSC to induce enhanced tissue repair and regeneration is possible but appears to be less likely in contrast to the immunomodulating effect of MSC.

At least two phase I studies are now underway in relapsing multiple sclerosis examining the safety and feasibility of intravenously administered MSC. These studies will also incorporate cellular immunology both in the peripheral blood and CSF. Furthermore, in-vivo methods of imaging to examine myelin injury and repair at the macromolecular level will also be employed to investigate the effect of MSC on tissue injury and repair. These studies are likely to provide the proof of concept supporting the use of MSC therapy in multiple sclerosis before undertaking larger multi-center initiatives.

Deriving Neural Progenitors from Human Embryonic Stem Cells for Brain Reparative Therapies

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Human embryonic stem cell (hESC)-derived neural progenitor (NP) transplantation offers great promise for treating brain disorders. Critical issues for NP therapy include the optimal purification and ideal state of NP differentiation for grafting. We examined methods for enrichment and differentiation of NP types (multipotent vs. neuronal restricted precursor [NRP]) from hESCs for transplantation after experimental stroke. To enrich for multipotent NPs, H7 or H9 hESCs (WiCell) were transfected with a construct containing the NP-specific transcription factor Sox3 (Sox3) human promoter driving a selectable marker (hSox3-GFP-hUbiquitinC-Neo). Stable lines were generated using antibiotic selection. Multipotent progenitors were enriched by fluorescence-activated cell sorting (FACS) for GFP, and NRPs by FACS using anti-polysialylated neural cell adhesion molecule (PSA-NCAM). Neural differentiation of hESCs yielded nestin/Sox3/ MAP2+ NPs in rosettes. Stably transfected hESC colonies expressed GFP only after neural differentiation; GFP+ cells formed Sox3+ rosettes and differentiated into neurons and glia; cells enriched after FACS for GFP generated NPs and neurons, but only generated astrocytes after prolonged culture. FACS for PSA-NCAM+ NRPs after 3-week differentiation yielded putative NRPs (>44%). Many NPs grafted into intact or infarcted forebrain were detected 1-2 weeks later and expressed nestin, Sox3 and occasionally □-III-tubulin. These findings suggest that promoter- or surface marker-based selection methods yield enriched NP populations that will be useful for optimizing transplantation therapy.

Embryonic Stem Cells Used for Intervertebral Disc Regeneration in an In-Vivo Model of Disc Degeneration

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BACKGROUND: There is currently no therapy to repair or restore degenerated intervertebral discs. Embryonic stem cells (ESC) can potentially grow indefinitely in vitro and differentiate into a variety of cell types. Therefore, ESC provide an attractive alternative to commonly used sources for deriving cells of various lineages for therapeutic purposes including cells capable of potentially producing nucleus pulposus. The notochordal cell is felt to be the origin of the intervertebral disc. As this cell is replaced by terminally differentiated chondrocytes, disc degeneration begins, most likely as a result of reduced proteoglycan production and subsequent loss of water content within the intervertebral disc.**PURPOSE:** To report on the potential of murine ESC and their capabilities to differentiate into notochordal cells in an In-Vivo rabbit model of disc degeneration.**METHODS:** A novel In-Vivo animal model of disc degeneration was developed by needle puncture of healthy discs in 16 New Zealand White rabbits. Rabbits were subjected to magnetic resonance imaging (MRI) pre-operatively and at 2, 4, and 8 weeks post-operatively. Once radiographically confirmed, degenerated disc levels where injected with pre-treated murine ESC labeled with a mutant green fluorescent protein (GFP). These cells were pre-treated to differentiate along a chondrocyte cell line. At 8 weeks intervertebral discs were harvested and analyzed with hematoxylin and eosin (H&E) staining, confocal fluorescent microscopy and immuno-histochemical analysis. Three intervertebral groups were analyzed: 1. control non-punctured discs (Group A, n = 32 disc), 2. experimental control punctured disc (Group B, n = 16 disc), 3. experimental punctured disc followed by implantation of ESC (Group C, n = 16).**RESULTS:** MRI imaging confirmed reproducible intervertebral disc degeneration at needle punctured segments starting at approximately 2 weeks. Post-mortem histologic analysis of group A intervertebral disc showed aged chondrocytes and almost complete disappearance of notochordal cells. Group B discs displayed intact annulus fibrosus and generalized disorganization of nucleus pulposus with increased bone formation . Group C discs showed viable new cartilage forming as well as notochordal cell growth. Fluorescent analysis was negative for groups A and B but revealed viable notochordal cells within experimental group C discs implanted with ESC. Of note, no inflammatory response as evidence of cell mediated immune response was noted in Group C discs. **CONCLUSIONS:** This study illustrates a novel reproducible model for the study of disc degeneration as well as disc regeneration using ESC. New notochordal cell populations were seen in ESC injected degenerated discs. The lack of immune response to xenograft implanted mouse cells in an immune competent rabbit model points to an immunologic sanctuary within the intervertebral disc.

Retinal Development

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Key Words: *Retina, Wnt pathway, neuron, development*

The retina represents a neural network which is one of the last tissues to develop in a human. A mature retina is also dependent on the maturation of retinal vasculature and the underlying retinal pigment epithelium. In understanding how this development occurs we may better understand what drives cellular differentiation and tissue organization. Evaluation of the normal and abnormal retina has identified many genes responsible for coordinating retinal development. Retinopathy of Prematurity (ROP) is characterized by abrogation of the normal retinal development and subsequent pathology. Investigations into ROP has allowed for analysis of certain genes and their role in normal retinal development. Similarly, many inherited vitreoretinopathies are characterized by abnormal retinal development. These vitreoretinopathies present a unique opportunity to better understand the role of certain genes and their importance in cellular differentiation. A Wnt receptor signal transduction pathway has been identified as playing a pivotal role in ROP and many inherited vitreoretinopathies. Mutations affecting the genes involved in this pathway have been associated with these disease states. Discovering the role of these genes and there temporal expression may lead to more successful treatments that utilize gene therapy and stem cells.

Stem Cell Biology Session II 10:20 am – 11:50 am

Development of Multipotent Stem Cells by Chemical Supplementations in Culture Medium
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At present, there are 3 major approaches to develop stem cells for clinical applications. Each of them has advantages and limitations. The embryonic stem cells (ES) are considered to have unlimited supply due to their infinite lifespan and high malleability to develop into many, if not all, cell types in the body. However, ES has the potential to develop into teratoma and its derivation carries high ethical burden and legal controversy. The development of patient-specific ES by somatic cell nuclear transfer may overcome immune rejection problem. However, the low success rate with the current technique is not practical and unethical because of the risk to women during egg donation. The second approach is to create induced pluripotent cells (iPCs) from somatic cells by gene transfer and ectopic expression of few key genes such as Oct4, Sox2, Klf4, and c-Myc (Takahashi et al.) or Oct4, Sox2, Nanog, and Lin28 (Yu et al.). The technique involves multiple insertions of retroviruses that might easily lead to mutation and tumorigenesis. The aberrant expression of Myc and Oct4 may also confer immortality and dysplasia and induce cancer.
The third approach is to develop cell culture technique to grow adult stem cells or multipotent stem cells. In fact, only these cells have been reported in practical reparative/regererative medicine thus far. Although, in general, adult stem cells have limited supply and developmental potential, there are many reports in recent years about the culture of multipotent stem cells with high proliferation potential from various tissues, such as bone marrow (MAPC), dermis (SKP), adipose (hMADS), and testis (maGSCs). These cells apparently require different growth factors, e.g. glial-derived neurotrophic factor (GDNF) for maGSCs or proper cell density to maintain self-renewal and pleuripotency.

Besides those genes listed above for iPC, several other genes might be targets for chemical modulation to achieve prolonged self-renewal and pleuripotency, e.g. stat3, telomerase, BMP, p16, Akt and Wnt pathways. Examples of chemicals that might modulate the expression of these genes are glycogen synthase kinase-3 inhibitor, 6-bromoindirubin-3'-oxime (BIO), fibronectin, bFGF, Noggin, Activin A, IL-6, and chemicals that change redox state of cells, e.g. N-acetyl-L-cysteine (NAC) and L-ascorbate-2-phosphate (Asc-2P),.

We have previously reported the development of several human adult stem cells, i.e. kidney, breast epithelial, pancreatic, liver, gastric and adipose-derived mesenchymal stem cells (AD-MSCs). All these cells expressed Oct4. Except the initial works with kidney and breast epithelial stem cells, our success may be ascribed to the use of a low calcium medium supplemented with NAC and Asc-2P. These 2 chemicals might modulate redox state of cells and regulate Oct4 and Sox2 expression. This culture technique has been also applied to the development of canine and porcine AD-MSCs, and canine kidney stem cells. All these MSCs were capable of differentiating into neuronal cells, besides mesenchymal lineages. Unexpectedly, the canine kidney stem cells were capable of differentiating into mesenchymal lineages, besides kidney tubular cells. Sustained growth of these cells required higher cell density and seems to involve stat3 expression. These studies provide further examples that multipotent stem cells can be developed by special cell culture technique with specific chemical supplementation.

Gastric Stem Cells: A Link to Gastric Cancer?
Deborah L. Gumucio, Ph.D., Tracy Qiao, Ph.D.

Epithelial stem cells in the stomach are responsible for the constant renewal of the epithelium and the generation of multiple gastric cell lineages that populate the gastric glands. However, gastric stem or progenitor cells have not been well-characterized due to the lack of specific markers that permit their prospective recognition. We identified an intestinal promoter (villin gene) that is active in a rare subpopulation of gastric epithelial cells and investigated whether these cells possess multi-lineage potential. A marked allele of the endogenous mouse villin locus was used to visualize single □-galactosidase positive cells located in the stem cell zone of a subset of gastric glands. A 12.4Kb villin promoter/enhancer fragment was shown to drive several transgenes, including EGFP, □-galactosidase and Cre recombinase in these cells in a pattern indistinguishable from the marked villin allele. Reporter gene activity was used to track these cells during development and to examine cell number in the context of inflammatory challenge while Cre activity allowed lineage tracing in vivo. Our studies demonstrate that these rare villin-marked epithelial cells are highly quiescent, but that interferon gamma administration results in their robust amplification. Lineage tracing studies confirm that these cells are multipotential and give rise to all gastric lineages of the gland. Recent microarray analyses of the isolated cells have resulted in the identification of additional potential markers for gastric stem cells. The ability to prospectively identify and manipulate gastric progenitors *in situ* represents a major step forward in gastric stem cell biology and has implications for gastric cancer.

Skeletal Stem Cells: The Cause and the Cure

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Keywords: *bone, cartilage, myelosupportive stroma, marrow adipocytes*

What are skeletal stem cells? From the pioneering work of Alexander Friedenstein in the 1960's, it is known that bone marrow is the home of two different stem cell populations: the hematopoietic stem cell, and the skeletal stem cell (also known as the "mesenchymal stem cell"). When single cell suspensions of bone marrow are plated at low density, a small population of cells becomes rapidly adherent (the Colony Forming Unit-Fibroblasts), and proliferates to form individual colonies of bone marrow stromal cells (BMSCs). Of these colonies, between 10-20% give to rise to bone, cartilage, myelosupportive stroma and marrow adipocytes upon in vivo transplantation. Recent work has shown that MCAM/CD146 isolates all CFU-Fs from bone marrow, and identifies pericytes that line marrow sinusoids as the skeletal stem cells.

What role do skeletal stem cells play in disease? Skeletal stem cells are central mediators in bone homeostasis, not only due to their ability to participate in bone formation, but also due to the fact that they control bone resorption. Consequently, any intrinsic change (mutation or epigenetic) or extrinsic change (in the microenvironment) that alters their biological activity will result in a skeletal disease or disorder. For example, Fibrous Dysplasia of Bone (FD) and the McCune-Albright Syndrome (MAS) are caused by post-zygotic activating missense mutations of Gs alpha, leading to overproduction of cAMP. Overactivity of Gs alpha in skeletal stem cells results in replacement of normal bone and hematopoietic marrow with structurally unsound bone and a fibrotic marrow that consists of misfunctioning CFU-Fs and BMSCs. When cells from the fibrotic marrow are ex vivo-expanded and transplanted in vivo into immunocompromised mice, they form an FD lesion with all of the pathological features found in the native lesion, thereby providing proof of principle of the skeletal stem cell as the causative agent of a skeletal disease. Consequently, skeletal stem cells are a target for the development of new therapeutic approaches

How can we use skeletal stem cells in regenerative medicine? There is ample evidence that ex vivo expanded BMSCs that maintain the subset of skeletal stem cells within them are capable of regenerating a bone/marrow organ when placed into segmental defects in conjunction with an appropriate scaffold, and a number of small clinical trials are under way around the world. In intact animal, cells administered via the circulation are rapidly trapped within blood vessels in the lungs, liver, and spleen and do not escape into the extravascular spaces. In the case of injury, small numbers of cells may be able to escape, but whether they can incorporate into the pre-existing 3D structure at a high enough level to have a biological effect is uncertain. However, there is current evidence that systemically administered BMSCs may have a "nursing" effect through their production of a large repertoire of growth factors and cytokines. The cells and their factors may promote survival, proliferation and differentiation of local stem/progenitor populations. Furthermore, there is emerging evidence that BMSCs are immunomodulatory and anti-inflammatory. Consequently, these cells may provide a unique therapy for the treatment of a large number of conditions for which current treatments are less than optimal.

Wnt/Beta-Catenin Signaling in Adrenocortical Stem/Progenitor Cells:
Implications for Adrenocortical Carcinoma

Gary D. Hammer, M.D., Ph.D.
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The long range objective of my laboratory is to understand the cellular and molecular mechanisms by which signaling pathways and downstream transcription factors coordinate the specification of adrenocortical cells within the adrenal gland in health and disease. Recent efforts examine the hypothesis that Wnt/beta-catenin signaling maintains the functional capacity of the adrenal cortex though the regulation of undifferentiated adrenocortical cell fate. Dysregulation of this system is predicted to result in abnormal adrenocortical growth and/or differentiation. Using cellular systems, mouse models together with genomic approaches with mouse and human adrenocortical carcinoma (ACC) samples, we aim to characterize the stem/progenitor cells of the adrenal cortex and uncover the mechanisms by which these cells are regulated by Wnt/beta-catenin signaling in normal adrenal growth maintenance and cancer.

In the adrenal cortex, Wnt/beta-catenin signaling is restricted to the subcapsular region. While these subcapsular undifferentiated adrenocortical cells are known to migrate centripetally into the cortical zones of the gland to populate the three zones of the adrenal cortex, the molecular mechanism underlying role of these cells in tissue homeostasis is poorly understood. We present data that support a role of Wnt/beta-catenin signaling in the self-renewal and multipotent properties of these adrenocortical cells in vivo. We also characterize mechanisms by which loss and gain of Wnt/beta-catenin signaling participate in the development of adrenal failure and ACC, respectively. ACC is an incredibly rare and routinely fatal disease with few effective treatments. Understanding the role of Wnt/beta-catenin signaling in adrenocortical cell fate will lay essential groundwork for future therapies that target this pathway and downstream genes that are found in the course of these studies to participate in adrenocortical stem/progenitor cell biology.

Stem Cell Therapy Session II 10:20 am – 11:50 am

Update on Hematopoietic Stem Cell Gene Therapy
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Keywords: *HSC, retrovirus, leukocyte, LAD*

Hematopoietic stem cells (HSCs) have emerged as attractive gene therapy targets for a wide range of diseases, including cancer, AIDS, and many inborn blood disorders. The greatest advantage of utilizing HSCs for gene therapy is that they are highly accessible cells as well as having a great potential for long lasting therapeutic impact. HSCs, by definition, can self-renew and differentiate into all hematopoietic lineages such as leukocytes, red blood cells and platelets. Although HSCs have been used to treat a wide variety of diseases through transplantation with reasonable success, the availability to obtain suitable donors is limited. In these cases, gene therapy has been explored, since the early 1990's, as a means to correct a variety of human genetic defects occurring in the hematopoietic lineages, especially when the correction of even a small percentage of cells could lead to improvement or reversal of the disease phenotype. Early gene therapy efforts focused on the use of gammaretroviral vectors, developed from murine leukemia viruses, to facilitate stable transfer of therapeutic genes into the genome of corrected HSCs. These early trials showed promise in treating several diseases, such as X-linked SCID, ADA-SCID, and CGD. However, the vectors were like a double-edged sword because the risk of insertional activation became a reality recently when leukemia and myeloproliferation occurred in X-SCID and CGD patients treated with gene therapy. Current studies in HSC gene therapy involve developing safer vectors and methodology. Towards this end, animal models have become invaluable tools to assess these developments. We use a canine model of the genetic disease leukocyte adhesion deficiency, termed CLAD, to examine the variables involved for successful and safe treatment of the disease. Dogs with CLAD suffer from recurrent bacterial infections due to a deficiency of the leukocyte integrin CD18. The deficiency leads to defective trafficking of the leukocytes to sites of infection. We explored the use of a novel retroviral vector system, based on the foamy virus, to transfer a copy of the normal CD18 gene into canine HSCs. We were able to get 5% to 10% correction of the peripheral blood leukocytes, which was sufficient to cure the dogs of their disease. Through genetic analysis of the vector integration sites, we have determined that these vectors carried a lessened risk of oncogenesis compared to the gammaretroviral vectors used in the aforementioned clinical trials. Continued development of these and other vector systems along with new technologies such as site-specific gene correction are likely to lead to new strategies for gene therapy of human genetic diseases using hematopoietic stem cells.

Making Blood from Embryonic Stem Cells: A Sanguine Future
Shannon L. McKinney-Freeman Ph.D. and George Q. Daley Ph.D.
Children's Hospital; Harvard Medical School; Harvard Stem Cell Institute, Boston, MA

Embryonic stem cells (ESC) represent a potential source of transplantable hematopoietic stem cells (HSC). A major goal of our laboratory is the derivation of potent HSC from human ESC that could be exploited in the clinic to treat hematopoietic malignancies and genetic disease. There are multiple projects ongoing in the laboratory that all contribute towards the realization of this goal: 1) the characterization of ESC-derived HSC (ESC-HSC) generated from murine ESC, 2) the identification and characterization of exogenous factors that promote the specification of blood progenitors in differentiating murine ESC, and 3) the derivation of patient specific human iPS cell lines. This presentation will be an overview of recent advances within the laboratory towards each of these projects.

Neural Crest Stem Cells in Development, Disease and Therapy
Jack Mosher, Ph.D.
University of Michigan, Ann Arbor, MI

During fetal development cells from the vagal level of the neural crest, including neural crest stem cells (NCSCs), migrate extensively to give rise to the neurons and glia of the enteric nervous system (ENS). Due to patterning differences along the neural crest, vagal level NCSCs are unique in their ability to migrate through the gut and to give rise to the diversity of neuronal subtypes found in the ENS. Defects in the migration of vagal NCSCs can lead to Hirschsprung disease, or aganglionic megacolon, a congenital defect that affects 1 out of 5,000 live births and is characterized by a failure to form the ENS in a variable length of the hindgut. This potentially fatal condition results in an inability to coordinate peristaltic movements of the bowel and is most commonly caused by mutations that reduce signaling through the glial cell line-derived neurotrophic factor (GDNF) or endothelin-3 (EDN3)

signaling pathways. Receptors for these pathways, Ret and Endothelin receptor B, respectively, are highly expressed by the NCSCs that give rise to the ENS. These signaling pathways interact to regulate the proliferation and migration of NCSCs and other neural crest progenitors that colonize the gut. Neural crest cells never migrate into the aganglionic portion of the gut in animals affected by *Ret* or *Ednrb* deficiency. These observations raise the possibility of improving the treatment of Hirschsprung disease by combining traditional surgical approaches with cell therapy in which NCSCs are transplanted directly into the aganglionic portion of the gut to generate enteric ganglia by bypassing the migration/proliferation defects. Consistent with this possibility, we and others have shown that NCSCs isolated from the fetal rodent gut can engraft and form enteric neurons after transplantation into the aganglionic region of the gut from rodent models of Hirschsprung disease. To translate these results into a potential therapy, we are currently attempting to derive and characterize vagally patterned human NCSCs from hES cells.

Panel- Stem Cell Ethics/Political Implications 1:00 pm – 2:00 pm

Stem Cell Research and Its Communication

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Paid by Wayne State University. Paid by Mary Ann Liebert, Inc. publishers to edit Stem Cells and Development

Keywords: *Ethics, Communication, Peer review, Regulatory issues*

Stem cell research and its communication have become, for better and worse, a matter of great public interest. The peer review process is still alive and well, but has taken a severe beating of late. This presentation will touch on some delicate issues relevant to the process, such as motivation behind paper submission, reviewer recommendation and selection. Stem cell researchers are a truly an international community, although communication is primarily in English. However, in spite of international recognition of the importance of ethics in the research and publication process, cultural and regulatory differences create a gulf of perspective that warrants consideration.

Stem Cell Disparities: Addressing Ethical Concerns Around Justice

Christine Gorka, M.S., M.A.,
St. John Health System, Southfield, MI

For many, the only ethical concern regarding stem cell (SC) research involves the moral question around using human embryos. However, there exist serious justice concerns relating to these promising therapies regardless of the stem cell source. New technologies are expensive. The latest enzyme therapy for Gaucher's disease costs $300,000 / year. The huge price tag makes this treatment unavailable for many. Likewise, economic barriers will make SC therapies inaccessible to some patient populations. In addition to financial hurdles, there is the growing recognition that barriers due to ethnicity also need to be addressed. This concern primarily arises from disparities observed in the transplant arena. According to statistics from UNOS, while whites and African Americans are almost equally represented on kidney waiting lists, whites receive transplants at rates twice as high as their African American counterparts. The disparity exists because minorities are less likely to find compatible tissue matches. Unlike the transplantation paradigm, which is constrained by organ availability, SC therapies will come from designed cell banks. Unless justice issues are addressed, the lack of ethnic diversity in the transplantation paradigm may be recreated in SC banks. Therefore, many minority patient populations will not benefit from these treatments. This presentation will address the ethical mandate of distributive justice by exploring what economic and biological access barriers need to be considered as stem cell research moves forward.

Drug Design/Development and Biotechnology Session 2:00 pm – 3:30 pm

Human Embryonic Stem Cells for Drug Discovery
Peter Sartipy, Ph.D.
Cellartis AB, Göteborg, Sweden

Improved technologies are urgently needed in order to develop more effective and safe new drugs. Undifferentiated or selectively differentiated human embryonic stem cells (hESCs) are expected to become important research tools for the pharmaceutical industries. The possibility to propagate hESCs and subsequently differentiate them into desired target cell types will provide a stable supply of cells for a range of applications. Improved *in vitro* models based on physiologically relevant human cells will result in better precision and more cost-effective assays ultimately leading to lower attrition rates and safe new drugs.

The research at Cellartis is focused on the development of functional cardiomyocytes and hepatocytes from undifferentiated hESCs. Molecular and functional characterization has demonstrated that the differentiated cells share similarities with their adult counterparts. These results lend support to the further development of assay systems based on these cells and novel opportunities and challenges will be discussed.

Umbilical Cord Blood-Derived Multi-Lineage Progenitor Cells (MLPC), a Model for Tissue Engineering and Drug Discovery
Barbara M. Tigges, Ph.D.
BioE, Inc., St. Paul, MN USA

Key Words: *progenitor cells, human umbilical cord blood, cellular differentiation, regenerative medicine, tissue engineering*

Regarding regenerative medicine, stem cells possess great potential within the tissue engineering and cellular therapy fields. They have been isolated from many sources such as but not limited to embryos, bone marrow, peripheral blood, umbilical cord blood, amniotic fluid, and adult tissues (ie. adipose and CNS). Their capability to differentiate into multiple lineages creates an attractive target for therapeutic purposes as well as pharma applications such as high-thruput screening [HTS]. MLPC are normal diploid cells derived from post-partum human umbilical cord blood that have the capacity to be extensively expanded. They are isolated from human umbilical cord blood with the use of a cell separation reagent, PrepaCyte®-MLPC. Further, characterizational analyses were performed such as CD marker profiles, molecular karyotyping, and microarray gene expression. These results revealed a novel stem cell with a normal karyotype that is highly uncommitted toward a particular lineage and is genetically and phenotypically distinct from a mesenchymal stem cell. MLPC are capable of being differentiated into cell types derived from and respresentative of each embryonic layer: mesoderm, ectoderm, and endoderm. These multiple cell types include adipocytes, osteoblasts, chondrocytes, neural subtypes, and respiratory epithelial cells. Quantitative PCR analysis determined the expression and upregulation of many differentiation-specific genes such as leptin (adipogenic) and alkaline phosphatase (osteogenic). Extensive functionality testing on the MLPC differentiated cell types will be our next focused objective.

High-throughput Biochemical profiling of DNA/protein complexes at Oct4 bound genomic regions.
Will Fairbrother Ph.D., Matthew Gemberling, Dean Tantin, Catherine Callister
Brown University,Providence, RI

Keywords: Chromatin IP, Oct4, transcription factor, ChIP-chip, biochemistry, genomic, high-throughput.

The transcription factor Oct4 is a key regulator of embryonic stem (ES) cell pluripotency and a known oncoprotein. We have developed a novel high-throughput binding assay called MEGAshift (microarray evaluation of genomic aptamers by shift) that we use to pinpoint the exact location, affinity and stoichiometry of the DNA-protein complexes identified by chromatin immunoprecipitation studies. We consider all genomic regions identified as OCT4-ChIP-enriched in both human and mouse. Compared to regions that are ChIP-enriched in a single species, we find these regions more likely to be near actively transcribed genes in ES cells. We re-synthesize these genomic regions as a pool of tiled 35-mers. This oligonucleotide pool is then assayed for binding to recombinant Oct4 by gel shift. The degree of binding for each oligonucleotide is accurately measured on a specially designed microarray. We explore the relationship between experimentally determined and computationally predicted binding strengths, find many novel functional combinations of Oct4 half sites and demonstrate efficient motif discovery by incorporating binding information into a motif finding algorithm. In addition, we use this high-throughput binding assay to profile the distribution of other DNA/protein complexes in the vicinity of Oct4. We believe that particular combination of factors may hold the key to

understanding stem cell specific gene expression. This method uncovers one such factor, Sp1, that appears to play a role in modulating Oct4 binding at natural Oct4 target sites in vivo.

Tissue Engineering Session 2:00 pm – 3:30 pm

Microfluidics for stem cell biology and therapy

Shuichi Takayama, Ph.D.
University of Michigan, Ann Arbor, MI

Keywords: *Microfluidics, Embryoid Bodies, Single Cell Manipulations, Laminar flow*

Many stem cell studies and cellular therapies require culture and manipulation of small numbers of highly environment-sensitive cells. The gap between the cellular microenvironment in vivo and in vitro, however, poses challenges for obtaining physiologically relevant responses from the stem cells. One of the reasons for this gap is because the fluidic environment of mammalian cells in vivo is microscale and dynamic whereas typical in vitro cultures are macroscopic and static. Another reason is that conventional cultures are often two-dimensional (2D) whereas physiological environments are 3-D.

There is increasing focus on unconventional interdisciplinary research opportunities to address biomedical challenges. One fruitful area for cross-fertilization is at the interface of biology and micro- and nanotechnology. Advances in the microelectronics industry and developments in nanotechnology have produced a variety of methods and technologies capable of fabricating and manipulating structures at the size scale of individual biomolecules and cells. At the same time, there has also been an unprecedented expansion of knowledge and capabilities in the field of biology; sequencing of the human genome and production of human embryonic stem cells to just name a few key discoveries. Many of these biological advances are at the cellular and macromolecular levels, which, in terms of length, are at the micro- and nanometer scales.

This presentation will give a few examples of efforts in our laboratory to develop programmable microfluidic systems that enable spatio-temporal control of both the chemical and fluid mechanical environment of cells. The presentation will also discuss methods to form and manipulate microscale 3D cell cultures within the microfluidic devices. The technologies and methods close the physiology gap to provide biological information otherwise unobtainable and to enhance cellular performance in therapeutic applications. Specific biomedical topics that will be discussed include microfluidic embryoid body formation and manipulation and studies of the effect of physiological and pathological fluid mechanical fluid stimulation on cellular responses.

Embryonic stem (ES) cell differentiation is often initiated by generation of embryoid bodies (EBs), which are three-dimensional cell aggregates formed by culturing ES cells on a non-adherent substrate. EB differentiation has been shown to recapitulate many aspects of early embryogenesis, resulting in the generation of a wide variety of cell types within EBs. It is well known that stem cell self-renewal or differentiation fates are particularly sensitive to the microenvironment. One crucial parameter that plays an important role in controlling the differentiation of ES cells is the regulation of EB size and interaction with other cells. Although efforts have been made to regulate EB size, conventional EB formation protocols are tedious or provide little homogeneity in the size of the resulting EBs. Here we describe a straightforward method to control the formation of EBs using microfluidic techniques.

Formation of EBs occurs spontaneously, in environments where cell-cell interaction dominates over cell-substrate interactions, through aggregation of ES cells maintained in close proximity to each other. What is required to form uniform size EBs, therefore, is to segregate ES cells into groups with defined number of cells in proximity to each other. The microfluidic EB formation method described here accomplishes this by using a two-layer device composed of two microchannels separated by a semi-porous polycarbonate membrane. When a suspension of ES cells is introduced into the upper chamber to form a monolayer of cells on the non-cell-adhesive semi-porous membrane, the cells synchronously self-aggregate to form EBs in which the size of the EBs is regulated by and proportional to the channel widths. The process of EB formation was imaged by time lapse photography and EB viability was confirmed through cell viability staining. We also demonstrate that the EBs thus formed can be removed from their microchannels and subjected to conventional differentiation conditions, including neuronal differentiation.

Alternatively, the EBs can also be subjected to various culture conditions inside the microchannels without disturbing their positions by simply exchanging media through the lower channel.

Additionally, one of the interesting capabilities of microfluidic cell culture systems is to utilize patterned laminar flows to control spatial delivery of cells, chemicals, and growth factors. When combined with microfluidic embryoid body formation, one can regulate the formation of embryoid bodies and spheroids where two different cell types can be patterned together in defined positions. This method provides a simple yet robust means of providing EBs with defined size and cellular compositions for subsequent use in conventional dish-based culture and differentiation protocols. The device designs and methods are also compatible with on-chip EB culture and differentiation, opening the way for more comprehensive “ES cell processing on a chip” to enable manipulations not possible in conventional cultures.

Fabrication of Functional Porous Structures for Tissue Engineering

[1]Pratapkumar Nagarajan, [1]Kathryn Abbott, [1]Sarang Deodhar, Donggang Yao, Ph.D. and [2]G. Rasul Chaudhry
[1]School of Polymer, Textile & Fiber Engineering, Georgia Institute of Technology, Atlanta, GA
[2]Department of Biological Science, Oakland University, Rochester, MI

A new method on the basis of the unique characteristics of immiscible polymer blends was developed for fabrication of porous scaffolds with controllable porous structures. In this method, immiscible polymers are first melt-blended to form a co-continuous structure with a characteristic phase size on the order of microns or smaller. The blend is then melt-processed using extrusion, injection molding or compression molding to create a useful outside geometry for scaffold applications. Finally, the blend is selectively dissolved to generate a porous structure. One salient feature of this new process is that the thermomechanical history of the blend during melt processing is controlled so as to create a desired porous structure regarding pore sizes, pore orientations and pore distributions.

A blend of polycaprolactone (PCL) and polyethylene oxide (PEO) was used as a model system. Both PCL and PEO are biocompatible polymers, and the latter is also water soluble. The two polymers have similar melting temperatures and desired rheological properties for forming a co-continuous blend morphology during mixing. A batch mixer was used for preparing a PCL/PEO blend which was further extruded, injection molded or compression molded into a 3-D outside geometry. It was found that the thermomechanical history during melt processing played a major role in the porous structure developed. Pore sizes varying from a micron to a hundred microns were created using different processing temperatures. Furthermore, by imposing a gradient thermal field during processing, a gradient porous structure was created. The biocompatibility of the resulting PCL scaffolds with a porosity of approximately 70% was verified using scaffold seeding experiments with stem cells and their derivatives. These scaffolds were found to promote the proliferation and growth of stem cells differentiated osseous cells.

Use of Synthetic Polymers for the Culture of hESCs

Luis G Villa-Diaz, Ph.D.[1] , Himabindu Nandivada[2],Jun Ding[3], Naiara C Nogueira-de-Souza[3] , Sue O'Shea[4], Jörg Lahann[2], Gary D Smith[1, 5-7]
Departments of Biology and Material Science[1], Chemical Engineer[2], Obstetrics and Gynecology [3], Cell and Developmental Biology[4], Urology[5], and Molecular and Integrated Physiology[6]; Reproductive Sciences Program[7]. University of Michigan, Ann Arbor, MI 48109-0617.

Human embryonic stem cells (hESCs) are pluripotent cells derived from human pre-implantation embryos and have enormous potential as a source of cells for cell replacement therapies and as a model for early human development. Current culture of hESCs on feeder-cell layers of mouse embryonic fibroblasts (MEF), human fibroblasts and/or autogenic fibroblasts has pitfalls that limit their use such as: time and effort spent maintaining a fibroblast cell line, potential source of contamination, limited integration with large-scale culture and genetic alteration of hESCs. Moreover, the co-culture system complicates the study of self-renewal and/or differentiation mechanisms of hESCs. Alternatively, feeder-free culture of hESCs on Matrigel, human laminin and fibronectin using media pre-conditioned by fibroblast is possible, although batch-to-batch inconsistency of these extracellular matrices due to the animal/human-derived nature of components is a risk involved in their use. Additionally, long-term stability of these protein-derived matrices must be questioned, therefore requiring continual batch-to-batch production of coated plates for hESC passage. Development of synthetic artificial matrices that support growth and proliferation of hESCs will be of great benefit, since their characteristics are fully defined. Artificial synthetic matrices can be modified and produced in large scales without batch-to-batch inconsistency, and they can be sterilized and stored for extended periods of time without loss of properties or stability. Hydrogels are hydrophilic polymer networks with physical characteristics similar to soft tissues. Six different types of hydrogels were tested for their ability to support feeder-free and long-term culture of undifferentiated hESCs: polyHEMA, polyMEDSAH, polyPEGMA, polyPLA, polyPLGA and polySPMA. As control Matrigel-coated plates and polystyrene tissue culture plates (Falcon plates) were used. Media pre-conditioned by irradiated-MEF and supplemented with bFGF was used to culture both H9 and BG01 hESCs on all surfaces mentioned above. No cell attachment was observed on polyPLA and polyPLGA plates. Cell attachment was observed on polyHEMA, polyPEGMA, polySPMA and polystyrene plates, but colonies spontaneously differentiated during the first or second passages. Cell attachment, colony growth and long-term proliferation of hESCs (over a period of eight months) were supported by Matrigel-coated plates and polyMEDSAH. Cells on both Matrigel-coated plates and polyMEDSAH plates have maintained undifferentiated proliferation for more than 30 continuous passages, and showed normal karyotype and expression of markers of pluripotency, including OCT3/4, SOX-2, SSEA-4, TRA-1-60 and TRA-1-81. These cells retained the potential to form derivatives of all three embryonic germ layers, as tested in vitro. To our knowledge this is the first report of long-term culture of hESCs on an artificial synthetic matrix – polyMEDSAH hydrogel-, and represents progress toward elimination of xenogeneic components in hESCs derivation and culture.

Matrix and Neural Crest: Tissue Engineering for the Periodontal Region
Tom Diekwisch Ph.D., Smit Dangaria, Yoshihiro Ito, Spencer Walker, Cameron Walker, Tianquan Jin, and Xianghong Luan

On their path from the free regions of the neural fold to their target tissues, neural crest cells are subjected to a multitude of factors and microenvironments presented by local substrates and surface conditions along their migratory path. The majority of these signals and structural cues are provided by a unique protein environment surrounding living cells, the extracellular matrix. The common origin of osteoblasts, cementoblasts, and odontoblasts from the craniofacial neural crest suggests similarities in gene expression patterns based on their common lineage in tandem with unique molecular signatures that specify the differentiation of individual target tissues. Here we are introducing a number of model systems to explore the role of factors and matrices on neural crest derived periodontal progenitors. Specifically, we demonstrate differences in terms of new collagen matrix synthesis between less differentiated dental follicle cells and lineage committed periodontal ligament cells as a result of inductive conditions. We also show that growth factors such as FGF2 and CTGF alone or in combination significantly enhance the expression levels of periodontal extracellular matrix proteins such as periostin, biglycan, and collagen III. Together, the studies presented here illustrate the importance of an extracellular matrix/stem cells interplay in craniofacial tissue engineering. Funding by NIH grant DE15425 to TGHD is gratefully acknowledged

Stem Cell Biology Session III 4:00 pm – 5:30 pm

The homologous transcription factors Oct1 and Oct4 are signal integrators and regulators of "stemness"
Dean Tantin, Ph.D.
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Keywords: *Oct4, Oct1, transcription, protein phosphorylation, mass spectrometry*

Oct4 is a known master regulator of the "stemness" phenotype in embryonic and germline stem cells. Although several pathways have been identified in the maintenance of adult stem cells and tumor stem cells, general molecular markers and master regulators of adult stem cell identity have not been identified. Oct1, which is expressed in most adult tissues, has a nearly identical DNA binding domain and identical DNA binding specificity as Oct4. Moreover, Oct1 and Oct4 share known targets. Although not stem cells, loss of Oct1 in murine fibroblasts leads to a decrease in the "stemness" gene expression signature. Loss of Oct1 leads to loss of stem cell markers in other systems. Senescent human fibroblasts have lower Oct1 levels compared to young cells. These results suggest that 1) stemness is a graded phenomenon, and 2) like Oct4, Oct1 can regulate aspects of stem cell identity.

We have identified in vivo Oct1 post-translational modifications following exposure of cells to stress stimuli. These modifications dynamically alter the ability of Oct1 to bind to complex sites. This mechanism is conserved in Oct4. Oct1 controls transformation in vitro and tumorigenicity in vivo through alterations in cellular metabolism. We are determining whether Oct4 acts in a similar pathway and whether "stemness" is in fact a metabolic phenomenon.

LINE-1 Retrotransposition in Human Embryonic Stem Cells
Jose L. Garcia-Perez Ph.D.[1], Sandra R. Richardson[1], K. Sue O'Shea[2], and John V. Moran[1].
[1]Departments of Human Genetics and [2]Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, USA.

LINE-1 elements (L1s) are abundant mobile genetic elements (*i.e.*, retrotransposons) that comprise ~17% of the human genome. An average human contains ~80-100 active L1s. Active L1s encode two proteins that are required for their mobility (*i.e.*, retrotransposition). L1 retrotransposition continues to sculpt the human genome and deleterious insertions into genes are associated with a variety of human diseases. Besides mobilizing their encoding mRNA *in cis*, the L1-encoded proteins also can function *in trans* to mediate the mobility of Short Interspersed Elements, certain non-coding RNAs, and some messenger RNAs, leading to the generation of processed pseudogenes. In total, these sequences comprise ~13% of human DNA. Thus, L1-mediated retrotransposition events are responsible for approximately one-third of our genome and appear to be responsible for at least ~1/1000 spontaneous disease-producing insertions in man. Despite these findings, relatively little is known about when and where L1 retrotransposition occurs during development. Here we will update our progress on using human embryonic stem cells as a developmentally relevant cell culture model to study the biology of LINE-1 retrotransposons.

Adipose Tissue as a Promising Source of Mesenchymal Stem Cells: Studies in a Canine Model

Manish Neupane[1], Chia-Cheng Chang[1,2], Matti Kiupel[1,3], *Vilma Yuzbasiyan-Gurkan*[1,4,5]
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Keywords: *Mesenchymal Stem Cells, Adipogenesis, Osteogenesis, Chonrogenesis, Gene Expression, Canine Model*

Mesenchymal stem cells (MSCs) hold great promise for regenerative and reparative medicine. MSCs have been isolated from various sources including bone marrow, adipose tissue, placenta and umbilical cord blood. Dog is a promising biomedical model for evaluation of novel therapies such as those employing stem cells in experimental and in spontaneous disease settings. This study is the first documentation of isolation and characterization of mesenchymal stem cells from canine adipose tissues. The canine adipose derived mesenchymal stem cells (cAD-MSCs) were isolated from subcutaneous adipose tissues, and expanded in a low calcium medium supplemented with N-acetyl-L-cysteine and L-ascorbic acid-2-phosphate. The cAD-MSCs differed from human adipose derived mesenchymal stem cells (hAD-MSCs) in terms of proliferation potential and their responsiveness to the conventional induction regimen. The differentiation of cAD-MSCs into osteoblasts and adipocytes was effectively achieved under modified conditions, by using laminin-coated plates and PPARγ ligands, respectively. The formation of micromass was sufficient to induce chondrogenesis, unlike hAD-MSCs, which require TGF-β. These cells displayed anchorage independent growth in soft agar, and their colony forming efficiency in plastic was comparable with human counterparts. The cAD-MSCs expressed genes associated with pluripotency while their differentiated progeny expressed appropriate lineage specific genes. With the establishment of these methods, this study lays the foundation for the development of the dog as a model system for regenerative medicine as well as for probing the role of stem cells in pathologic processes such as cancer.

Keynote Speaker 6:30 pm – 7:30 pm

Mouse Genetics: the Changing Landscape

Andras Nagy, Ph.D.
Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

The ability to modify the mouse genome by utilizing ES cell-based technologies has already had an unprecedented impact on our understanding of developmental and disease processes. The random nature of transgene insertions and “fixed structure” targeted or gene trap mutations, however, limits the discharge of the full potential of these genetic approaches.

Here we present a generalized system utilizing the properties of PhiC31 integrase and Flp recombinase to obtain a clean replacement of a specifically designed genomic site (docking site) with any desired transgenic element. A combination of positive and negative selection systems assist the replacement procedure, eliminating cells which have not undergone the designed genomic insertion. Furthermore, the selection system removes itself after identification of the site-specific integration of the incoming vector. As a consequence, only the desired transgenic element (replacer) has been left at the defined position.

This strategy has been implemented from the production of simple transgene insertions into a defined and characterized genomic insertion site to the replacement of targeted or gene trap insertions with vectors providing new functionalities to mutant alleles. The flexibility provided by this clean replaceable system led to its introduction into the high throughput gene targeting and gene trapping effort of the Genome Canada supported NorCOMM project. This endeavor is now at the stage of initiating the targeting of 2000 genes and in the process of deriving 144,000 gene trap lines.

The replacers fall into two categories; one is the universal replacers, such as known reporters, positive or negative selectable markers, toxins, transcription factors for drug inducible gene expressions, etc. The second set of replacers are trapped or targeted gene-specific; such as those used to change a primary null mutation into a Cre recombinase conditional allele, mutant cDNA knock-in. The vectors will be collected in a “toolbox” and will be available for the research community to perform the secondary change on the primary ES cell lines or, alternatively, the change could be provided by future core facilities.

POSTER SESSION ABSTRACTS

POSTER #1.

A Disintegrin and Metalloproteinase 17 (ADAM17) Activates EGFR-dependent Pi3K/Akt Activity in Adult Subventricular Zone Progenitor Cells and Contributes to Proliferation Following Stroke

Mark Katakowski[1], *Alexandra Szalad*[1, 2], Zheng Xu Guang[1], and Michael Chopp[1, 2]
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Keywords: *ADAM 17, SVZ, proliferation*

Background & Objective: A disintegrin and metalloproteinase 17 (ADAM17, a.k.a TACE) is a potent sheddase for multiple epidermal growth factor (EGF) pro-ligands. In the mammalian brain, EGF stimulates expansion of transit-amplifying neural progenitor cells within the subventricular zone (SVZ) and increases neurogenesis. In this study, we investigated whether ADAM17 could enhance EGF activity and proliferation of SVZ progenitor cells. Furthermore, we tested whether ADAM17 played a role in stroke-induced expansion of the SVZ progenitor pool.

Experiments: To this end, we tested whether ADAM17 could enhance EGFR and/or Pi3K/Akt activity and proliferation of SVZ cells in vitro. Subsequently, we measured ADAM17 expression and activity within the SVZ of normal and stroke animals, and determined whether antagonism of ADAM17 activity altered stroke-induced SVZ proliferation in adult rat.

Results: Over-expression of ADAM17 in primary cultured adult rat SVZ cells increased proliferation in vitro. Western blot indicated over-expression of ADAM17 lead to increased phosphorylation of EGFR and Akt, and that enhanced Akt phosphorylation could be ablated with treatment of the EGFR inhibitor AG1478 or the Pi3K inhibitor LY294002, indicating Akt activity was EGFR/Pi3K-dependent. ELISA of media conditioned by cultured SVZ neurospheres showed ADAM17 over-expression increased soluble EGF, signifying enhanced EGF-sheddase activity. In vivo, ADAM17 transcription, expression and proteolytic activity were significantly increased within the SVZ of adult rats subjected to middle cerebral artery occlusion (MCAo). Furthermore, 7-day ventricular micro-infusion of the ADAM17 inhibitor TAPI-2 reduced bromodeoxyuridine (BrdU) incorporation and Ki67 immunoreactivity in SVZ cells, indicating ADAM17 proteolysis is necessary for proliferation of adult SVZ cells following cerebral stroke.

Conclusions: SVZ cells express several substrates for ADAM17, including several EGFR pro-ligands that are known to stimulate SVZ proliferation and enhance stroke-induced SVZ neurogenesis. Our data suggest ADAM17 sheds EGFR-binding ligands in cells of adult rat SVZ, and that this proteolysis contributes to proliferation of SVZ progenitors. Furthermore, our data suggest ADAM17 proteolysis is a vital component of subventricular neurogenesis following stroke. Based upon our in vitro findings, we suggest ADAM17 may enhance stroke-induced SVZ proliferation via activation of EGFR-binding ligands. However, confirmation of this hypothesis requires further study. Taken together, our findings indicate ADAM17 proteolysis promotes stroke-induced SVZ neurogenesis and thus, may represent a therapeutic target for stroke recovery.

POSTER #2.

MRI Investigation of Angiogenesis of Embolic Stroke in Rat After Cell Therapy

A. Bosomtwi[1,3], Q. Jiang[1,3,4], Z.G. Zhang[1,4], G.L. Ding[1], L. Zhang[1], L. Li[1], R.L. Zhang[1], L. Wang.[1], H. Meng[1], J.R. Ewing[1,3,4], J. Hu[5], A.A Ali[2], Q.J. Li[1], R.A. Knight[1,3,4], and M. Chopp[1,3,4]
Henry Ford Hospital, Departments of Neurology[1], Radiology[2], Detroit, MI and Department of Physics[3], Oakland University, Rochester, MI and Departments of Neurology[4], MR Center[5], Wayne State University, Detroit, MI.

We investigated transplanted cells derived from the subventricular zone (SVZ) of the adult rat labeled by superparamagnetic particles into adult rats after stroke. Our results indicate that MRI identifies angiogenic tissue after SVZ treatment, and transplanted cells selectively migrate to the ischemic parenchyma, primarily to the angiogenic region. MRI measurements of various parameters distinguished angiogenic from non-angiogenic ischemic tissues. Angiogenesis and migration of transplanted cells in the host brain were confirmed using histochemical staining and were compared to MRI data. Our data suggest that MRI can be used to detect migration, distribution of grafted cells and cell induced angiogenesis in the host brain in living animals.

POSTER #3.

Quantitative Detection of Microvascular Density Changes after Stroke in Rat

A. Bosomtwi[1,3], Q. Jiang[1,3], G.L. Ding[1], L. Zhang[1], Z.G. Zhang[1,3], M. Lu[2], J. Ewing[1,3], M. Chopp[1,3]
Department of Neurology[1], Biostatistics and Research Epidemiology[2], Henry Ford Health Science Center,Detroit, Michigan, Department of Physics[3], Oakland University, Rochester, Michigan

We investigated vascular remodeling after stroke using MRI microvascular density (MVD) measurement and gold standard immuno-histochemistry staining. We demonstrate that MRI MVD detect the microvascular status of brain tissue with different ischemic damage. The MVD measured by MRI was highly correlated with histological measures of MVD. Our data demonstrate that MRI MVD measurement can quantitatively evaluate microvascular remodeling after stroke.

POSTER #4.

Isolation of Multipotent Human Umbilical Cord Blood Stem Cells

Jaime Brozowski, G. Rasul Chaudhry
Department of Biological Sciences, Oakland University, Rochester, MI, USA

Keywords: *adult stem cell, cord blood, umbilical cord*

Recent advancements in stem cell research provide promising ways and sources to isolate stem cells with vast proliferative and differentiation abilities. However, among the adult stem cells, only the cord blood stem cells have shown greater proliferative and lower immunogenicity. We hypothesized that umbilical cord can not only be more abundant and primitive, but also possess greater proliferative and differentiation potentials, as compared with the other sources of adult stem cells. To test this hypothesis, we are investigating the content of umbilical cord to isolate multipotent stem cells. We report here the reproducibility of the isolation of stem cells from umbilical cord blood samples. These isolated cell colonies developed after 2-3 weeks with two different physical properties within the same passage. One type of isolated colonies produced a monolayer with rapidly expanding fibroblastic spindles, where as the other colony type possessed a three-dimensional, circular shape without any cellular projections. Further characterization and differentiation potential of these isolates is being investigated.

POSTER #5.

Toxicity of Gold Nanoparticles in Embryonic Stem Cells

Jaime Brozowski, Ardit Kacorri, Chris Fecek, G. Rasul Chaudhry
Department of Biological Sciences, Oakland University, Rochester, MI, USA

Keywords: *nanomaterial, cytotoxicity, gold nanoparticles*

Nanomaterials, characterized as any particle less than 100 nm, are attractive for use in technological advancements not only for their small size, but also for their unique properties. Recent rapid progress in nanotechnology have led to wide spread applications of nanomaterials from industry to medicine. With increasing utilization and exposure to nanomaterials, it is essential to investigate their possible adverse effects on human health. Nanoparticles of nonreactive metals, such as gold and silver, exhibit exceptional optoelectronic properties and have gained considerable attention for various uses including biological optical imaging, sensing applications, and drug therapy. However, little is known regarding the mechanism of action of nanomaterials in the human body. In this study, we investigated the effects of gold nanoparticles on embryonic stem cell self-renewal, proliferation, and differentiation. Murine embryonic stem cells (ESCs) were treated with a heterogeneous mixture ranging from 10 to 100 nm, homogeneous mixture of 2 nm, and homogeneous mixture of 5nm of gold nanoparticles at concentrations ranging from 0.1 μg/ml to 50 μg/ml, 0.001 μg/ml to 0.50 μg/ml, and 0.01 μg/ml to 5.0 μg/ml, respectively. The results showed that the heterogeneous mixture caused toxicity at higher concentrations (>50 μg/ml). However, at lower concentrations (>1 μg/ml) heterogeneous mixtures promoted growth and the colonies were relatively larger in size than the control. ESCs treated with homogeneous mixtures of 2 nm and 5 nm nanoparticles caused a reduction in colony size and cell proliferation. Higher doses (>5 μg/ml) of 2 nm and 5 nm gold nanoparticles displayed cytotoxic effects and cells became necrotic when exposed to gold nanoparticles. The doses of gold nanoparticles that did not cause cytotoxicity had no effect on the pluripotency and self renewal of ESCs. Furthermore, the expression of ESC specific genetic markers, such as Oct 4, was not affected, and therefore, suggests that the stemness of these cells was not compromised. These investigations suggest that gold nanoparticles may cause embryotoxicity or teratogenicity and may also interfere with early

developmental processes, though further investigations of the molecular mechanisms of gold nanoparticle interactions in embryonic stem cells are warranted.

POSTER #6.

Stroke promotes DCX-expressing SVZ cells to neuronal lineage cells based on electrical properties and single-cell gene expression profiles

Ben Buller[1,2], Xian Shuang Liu[1], Xue Guo Zhang[1], Rui Lan Zhang[1],
Sara R Gregg[1], Ann Hozeska-Solgot[1], Michael Chopp[1,2], Zheng Gang Zhang[1]
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Stroke increases neuroblasts in the subventricular zone (SVZ) of the lateral ventricle and these neuroblasts migrate towards the ischemic boundary to replace damaged neurons. However, it has not been examined whether or how ischemia may affect the electrophysiological properties and gene expression profiles of migrating neuroblasts. Using acute brain slices from transgenic mice which expressed green fluorescent protein (GFP) concomitantly with doublecortin (DCX), a marker for migrating neuroblasts, we devised a novel strategy to record electrophysiological characteristics while simultaneously analyzing the expression of 15 genes in a single DCX-expressing (DCX-EGFP) cell. Using whole-cell patch clamp recordings, we found that DCX-EGFP positive cells in the non-ischemic SVZ had a mean rest potential (RP) of -23 ± 5.9 mV (n=26 cells) and did not show Na^+ current, characteristic of immature neurons. However, DCX-EGFP positive cells in the ischemic SVZ exhibited a more hyperpolarized mean RP of -53.9 ± 17.7 mV (n=30 cells) and displayed Na^+ current, indicative of more mature neurons. Using single cell multiplex RT-PCR, we then examined simultaneous gene expression in a single DCX-EGFP cell captured by the patch clamp. All DCX-EGFP positive cells expressed DCX, confirming the specificity of this cell population. DCX cells in the non-ischemic SVZ more highly expressed neural progenitor marker genes, Sox2, nestin and Mash1, but not mature neuronal marker genes. In contrast, DCX cells in the ischemic SVZ expressed tyrosine hydroxylase and calretinin, mature neuronal marker genes. Real-time RT-PCR analysis revealed that stroke significantly upregulated a Na^+ channel subunit gene, Nav1.2. Together, these data suggest that stroke promotes migrating neuroblast differentiation to more mature neurons.

POSTER #7.

One Year Follow Up of Autologous Muscle Derived Stem Cell Injection Pilot Study to Treat Stress Urinary Incontinence

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Key words: *Stress urinary incontinence, stem cell, muscle, urethra*

There are over 200 million people worldwide with incontinence, a condition that is associated with a reduced quality of life. Stress urinary incontinence (SUI) has been reported as the most common type of urinary incontinence. Stem cell therapy for the regenerative repair of the deficient sphincter for treatment of SUI has been at the forefront of incontinence research. The regenerative response of muscle derived stem cells (MDSCs) has been shown to be superior to myoblasts in a myocardial infarct model. **Materials and methods:** We hereby report one year follow up on 8 women in the first North American safety and feasibility trial in which SUI was treated with MDSCs injections. Eight women at Sunnybrook Health Sciences Centre in Toronto, CA entered this trial. Patients age 18 and over with primary symptoms of SUI, normal detrusor activity on filling cystogram and bladder capacity over 200 ml were eligible. All subjects had no improvement in symptoms for at least 12 months, and failed prior non-invasive treatment such as biofeedback, behavior modification or electrical stimulation. A biopsy was done using a percutaneous needle technique. Muscle tissue was obtained from the thigh and shipped to the cell processing facility in Pittsburgh, PA, USA. Once received in the cell processing facility, the biopsy was processed to isolate desirable cells and expand their number to the target dose. Three to five weeks later the frozen cells were shipped backed to Toronto and 18-22 x 10^6 MDSCs were injected in the outpatient clinic with local anesthesia. Follow up visits were performed at 1, 3, 6 and 12 months after injection. Pre- and post-injection pad weights, bladder diaries, and quality of life measures were utilized to assess outcome.**Results:** Eight women with a mean age of 54 years (range 42 – 65 years) at the date of injection were treated. Three subjects withdrew from the study after 1 month post-injection follow up. Improvement in SUI was seen in the remaining 5 women demonstrated by diary and pad weight test, with one subject achieving total continence. Onset of improvement was between 3 to 8 months after injection. Cure or improvement continued at a median of 10 months. The subject reporting total continence maintained the response at the 12 month

study endpoint. Two subjects demonstrated a reduction in incontinent episodes and pad weight tests of approximately 50% from baseline observations. Both received a second injection between 4 and 8 months after the first, with some additional improvement noted. However, these two subjects continued to have stress episodes and positive pad weight tests at all follow up visits. No serious adverse events were reported. Subsequent midurethral tape placement and outcome was not negatively impacted upon by previous MDSCs injection. The periurethral injection route or transurethral route utilizing a longer 10mm needle appeared to be associated with increased success.**Discussion:** We concluded that pure cellular therapy with MDSCs may lead to durable objective and subjective improvement of SUI. Onset of improvement is delayed following injection, suggesting that restoring muscle function may be the mechanism of action unlike standard bulking agents. Deeper delivery of MDSCs into the external sphincter appears to be important for successful outcome. However, the appropriate dose of cells has yet to be determined and investigations using true randomization, blinding and dose escalation designs are required.

POSTER #8.

Tsc-mTOR Pathway Maintains Quiescence and Function of Hematopoietic Stem Cells by Repressing Production of Reactive Oxygen Species

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It is suggested that hypoxia in the stem cell niches is critical to maintain the hematopoietic stem cells (HSCs) but the underlying mechanism is unknown. Here we report that genetical removal of Tsc complex, which is activated by hypoxia in HSCs and an important negative regulator of the mTOR pathway, drives the HSCs from quiescence into rapid cell cycle and increased apoptosis, with a net result of high frequency of HSCs in bone marrow (BM) and extramedullary hematopoiesis in spleens. Deletion of *Tsc1* in HSCs dramatically reduced their functions as revealed by serial and competitive BM transplantation and these defects are cell intrinsic. Increased mTOR activity results in upregulated mitochondrial biogenesis and dramatically increased levels of reactive oxygen species (ROS). In vivo treatment with ROS antagonist restores HSC number and their in vivo functions. Our data demonstrate that TSC-mTOR pathway is required to maintain the quiescence and functions of HSCs by repressing ROS production. This might be a molecular link between hypoxic niches and 'stemness'.

POSTER #9.

Injury Induced Pluripotential Daughter STEM Cell Migration in the Mammalian Hypothalamus

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Keywords: *Median eminence, nitric oxide Synthase, endocrine hypothalamus, migratory neuroblasts, nitric oxide*

Current research and research training in recent years has not emphasized combined correlative Scanning-Transmission electron microscopy coupled with immunoelectron microscopy and light microscopic immunocytochemistry as a means of stem cell analysis. Using such techniques we believe that we bring relatively rare skills that will help shed light on the study of neuronal stem cells, their daughter neuroblasts, neuritic projections and their over all migratory abilities in the endocrine hypothalamus. One of the many basic questions that this investigation confronts is, can stem cells or their prodgeny cross lineage boundaries and undergo phenotypic differentiation into migratory neurons? Of equal importance is the question whether the up-regulation of nitric oxide Synthase, following trauma to the neural lobe (selective neurohypophysectomy) is converted to the molecule nitric oxide (NO) by triggering the conversion of L-Argimine to NO. NO is currently felt to play a privotal role in this fundamental process coupled with great plasticity in this part of the endocrine hypothalamus. Finally can this process be reversed by the antagonist of NO, namely nitroarginine and to what extent? Further studies are now employing nitroprusside and glutamate both donors of NO to accelerate the migratory process of neuroblasts and their neurits that reach the cerebral ventricular (Lumen) during and after trauma.

POSTER #10.

Stress Enzyme Activation Tips the Balance from Pluripotencey to Differentaiton in Embryonic and Trophoblast Stem Cells

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Keywords: *ES cells; pluripotency; transcription factors; stress enzymes*

Embryonic stem (ES) cells and placental trophoblast stem (TS) cells derive from the first lineages of the mouse and human preimplantation blastocyst. Preimplantation embryos may encounter stress in vivo (such as nutritional stresses) and in vitro, such as those encountered during in vitro fertilization (IVF) or stem cell cloning procedures. We have found that cellular stressors downregulate the expression of transcription factors that maintain potency in ES and TS cells. A stress-response mechanism shared by these cell types activates those enzymes which induce the downregulation of pluripotency-maintaining transcription factors and through the release of inhibition, lead to the upregulation of differentiation-mediating factors. The kinetics, stress enzymes, and transcription factors involved are different for ES and TS cells. In ES cells, stress downregulates OCT4, SOX2, and NANOG proteins with similar kinetics although these protein levels rebound by 24hr. At 24hr, stress upregulates levels of GATA6 protein, suggesting that stress may favor differentiation. In TS cells, stress induces the upregulation of HAND1 through the actions of SAPK, and the downregulation of ID2 via AMPK activation. In contrast to ES cells, continuous stress does not result in a rebound of these protein levels. Since HAND1 upregulation and ID2 downregulation are necessary to produce the first placental hormone detected after implantation, we conclude that stress induces differentiation in TS cells through the action of AMPK and SAPK. These findings suggest strategies for optimizing the health of preimplantation embryos, whether during IVF for subsequent reimplantation in the host, or during the isolation and maintenance of high quality ES cells.

POSTER #11.

Regeneration of Nucleus Pulposus using ESC-Derived Chondroprogenitors in a Rabbit Disc Degeneration Model

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Keywords: *Chondrogenesis, tissue engineering, proteoglycan quantification, nucleus pulposus*

Spinal disc degeneration is a leading cause of back pain and discomfort in the United States. As a result of degeneration, intervertebral discs lose their functionality and ability to absorb shock. This may cause nerve compression and impingement leading to mild to severe back pain and missed work days. Such consequences will have a major negative impact on business productivity and, ultimately, the economy. Currently, no therapy exists to effectively regenerate the nucleus pulposus and restore the functionality of the degenerated disc. Embryonic stem cells (ESCs) offer an attractive potential treatment due to their pluripotency and their ability to proliferate indefinitely. Our earlier studies have shown that ESCs can be differentiated into chondrocytes that produce specialized cartilaginous tissues, such as hyaline cartilage *in vitro*. The goal of this study was to investigate the potential of the ESC-derived chondroprogenitors to regenerate the nucleus pulposus in the disc. We first investigated the differentiation of chondroprogenitors into chondrocytes *in vitro*. The results showed that ESC-derived chondrocytes exhibited typical morphological feature based on the LM and H & E analysis. The differentiated cells were positive for aggrecan and Col II based on the immunostaining using specific antibodies. Our procedure to differentiate chondroprogenitors yielded over 75% mature chondrocytes. When the chondroprogenitors were implanted into degenerated rabbit discs (n=4), the donor cells survived for 8 weeks based on post implantation analysis of cryosections of the implanted discs. The confocal analysis of the cryosections further showed that the implanted progenitors proliferated and integrated into the disc tissue.
These results suggest that chondrogenic derivatives of ESCs may be used to repair and regenerate the nucleus pulposus and may restore the functionality of damaged intervertebral discs.

POSTER #12.

The Effects of Polycyclic Aromatic Hydrocarbon on Adult Human Epithelial Liver Stem Cell Line
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Keywords: *Gap junctional intercellular communication (GJIC), Polycyclic aromatic hydrocarbons (PAHs),, Human liver epithelial stem cell*

Background: Cigarette smoke contributes to many human pathologies including cancer and cardiovascular disease. Polycyclic aromatic hydrocarbons (PAHs) is one class of compounds in this complex mixture of combusted by-products of tobacco. Although the most abundant PAHs in cigarette smoke are methylated anthracenes and phenanthrenes, the toxicity of these compounds has not been extensively studied and the effects of these PAHs on cell signaling relevant to cancer is not known. Gap junctional intercellular communication (GJIC) maintains tissue homeostasis and the blockage of GJIC by chemicals and other mechanisms has been shown to be the underlying mechanism for tumor promotion and other pathologies. The stem cell characteristic of a clonally derived human liver epithelial stem cell HL1-1 was indicated by the expression of alpha-feto protein (AFP) and vimentin, high proliferation potential, the ability of AIG and differentiation and the lack of GJIC. When grown in a modified MEM, the cells exhibit high level of GJIC.

Methods: The effects of 1- and 2-methylanthracene (1-MeA, 2-MeA) on cell signaling pathways, including GJIC, has been extensively characterized in a rat liver epithelial stem cell line. In this study, we compared known signaling effects in these rat cells with our human liver epithelial stem cell line, HL1-1. GJIC was measured using the scrape loading dye transfer (SL/DT) technique, and Western blots analysis were used to measure other signaling enzymes, including mitogen activated protein kinase (MAPK) and connexin (Cx) levels. Selected inhibitors of cell signaling enzymes were used for identifying causal links with GJIC.

Results: The 1-MeA, which contains a bay-like region, inhibited GJIC in a dose and time dependent manner, while the 2-MeA, which contains no bay-like region, had no effect. Inhibition of GJIC by 1-MeA was dependent on activity of phosphatidylcholine-specific phospholipase C (PC-PLC) and also prevented by pretreatment with H89 (inhibitor of protein kinase A (PKA)) or resveratrol (known polyphenolic antioxidant and anti-tumor compound). Inhibition of MAPK/ERK pathway did not affect 1-MeA-induced inhibition of GJIC. Although MAPKs were activated, Western blot analyses indicated no change in connexin phosphorylation status of Cx43. Similar to TPA, Cx43 was delocalized from the plasma membrane in response to 1-MeA, but not in response to 2-MeA. In addition, inhibition of PC-PLC by D609, PKA by H89 and antioxidant effect by resveratrol prevented the delocalization of Cx43.

Conclusions: Our results indicate that the response of HL1-1 cells to PAHs was very similar to the rat liver epithelial stem cell line. PC-PLC and PKA are some of the key signaling enzymes involved in PAH-induced regulation of GJIC. Additionally, resveratrol, a red wine phenolic, might have a specific antioxidant effect that prevents PC-PLC-dependent deregulation of GJIC by 1-MeA.
Support: NIEHS grant #R01 ES013268-01A2 to Upham.

POSTER #13.

Axolotl Limb Regeneration: Implications for Bone Regeneration Therapies
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Keywords: *Axolotl, Limb regeneration, Blastema cells, Bone marrow stromal cells, Bone regeneration*

Osteoporosis, a notorious bone disease, leading to the progressive loss of bone mass, increased bone fragility, and bone fractures, has been a major health problem suffered by aging people. However, successful clinical therapies have not been well-established. The goal of our study was to advance the bone regeneration therapies through two parallel research platforms: (1) Axolotl limbs were used as regeneration-competent models to explore cellular and molecular mechanisms of regenerating musculoskeletal tissues. All-trans retinoic acid (RA) was used as a model drug because it up-regulates sonic hedgehog for anteroposterior patterning in limb development and regeneration. On day 9 post-amputation, RA-loaded poly(lactic-co-glycolic acid) (PLGA) scaffolds were implanted in bioreactor spaces created either in dorsal fin tunnel (Fin), intra-peritoneal (IP), or in-between apical epithelial cap and amputation plane (AEC-AP). After two months post-implantation, histology studies demonstrated that the radius and ulna of amputated forelimbs duplicated for axolotls with either Fin or IP implants, suggesting the efficient release of RA from implants. By contrast, blastema formation was inhibited when the scaffolds were implanted in AEC-AP, indicating that the integrity of extracellular matrix is essential for normal dedifferentiation, proliferation, and migration of blastema cells. These observations imply that no healing of bone gap above critical size in axolotls could be also caused by the insufficient accumulation of mesenchymal stem cells. Further comparative studies on limb regeneration and axolotl bone repair will provide new insights into the tissue remodeling mechanism for a bone regeneration-permissive or -inhibitory environment. (2) As a parallel *in vitro* model, rat bone marrow stromal cells (BMSCs) were cultured on porous scaffolds based on PLGA, polycaprolactone (PCL), poly(ethylene glycol)-block-

poly(propylene glycol)-block-poly(ethylene glycol) (PEO-PPO-PEO), and their combinations. Cellular proliferation over a 15-day culture period was examined by WST-1 assay. For PLGA/PCL scaffolds with ~180-300 μm pore size, there was no significant difference in cell density on scaffolds with X_{PCL} from 0% to 80%, while the cell density slightly decreased with increasing X_{PCL} from 80%, 90%, to 100%. However, the viable cell number on PCL scaffolds with pore size >300 μm was comparable to that on PLGA scaffolds with ~180-300 μm pore size. Meanwhile, gradually increased cell density was observed for PCL scaffolds with pore size ranging from <180, ~180-300, to >300 μm, suggesting that sufficient large pore sizes are essential for BMSC migration and nutrient transportation. Furthermore, the cell density on PCL/PEO-PPO-PEO scaffolds decreased with increasing $X_{PEO-PPO-PEO}$ due to the "nonfouling" properties of "-PEO-" segments, though cell suspension could be directly seeded on these scaffolds without pre-wetting. Electron micrographs of BMSC/scaffold constructs showed that the cells attached well on scaffold surface with numerous pseudopods and some cells appeared to migrate into microspores. These results suggest that PLGA and PCL/PLGA composites having controlled pore size, pore interconnectivity, and mechanical strength can support BMSC attachment and proliferation. Further studies on BMSC culture with the presence of axolotl blastema protein extracts will be conducted to gain a thorough understanding of BMSC phenotypes.

POSTER #14.

Stress Differentially Regulates Phosphorylated and Nonphosphorylated CDX2 Serine 60 Protein Subpopulations in Mouse Blastocysts

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CDX2 is a transcription factor necessary for determining placental trophoblast stem cell (TSC) lineage in the blastocyst. In intestinal epithelial stem cells, phosphorylated CDX2 Serine 60 (Ser60) is transcriptionally less active and expressed in the proliferative compartment and nonphosphorylated CDX2 Ser60 is more active and expressed in the differentiating compartment, although both enter the nucleus and bind DNA. Thus, the phosphorylation state of CDX2 Ser60 may predict the function of CDX2 in the blastocyst. In mouse blastocysts, we detected nonphosphorylated and phosphorylated CDX2 Ser60 protein at the predicted size by immunoblot and both were detected in nuclei of trophectodermal cells of the placental lineage. As in the small intestine, phosphorylated CDX2 was detected at higher levels in the proliferative polar trophectoderm and nonphosphorylated CDX2 was detected at higher levels in the differentiating mural trophectoderm. Stress tends to cause differentiation in oocytes, embryos and stem cells (Rappolee, 2007), and stressed blastocysts have increased nonphosphorylated CDX2 and decreased phosphorylated CDX2. The data suggest that both CDX2 Ser60 protein subpopulations are present in the early blastocyst and may mediate separate functions in the early placental lineage. The data are consistent with the hypothesis that stress causes differentiation by increasing nonphosphorylated CDX2 and decreasing phosphorylated CDX2. The existence of both phosphorylated and nonphosphorylated CDX2 Ser60 in the blastocyst during the first establishment of the placental TSC lineage may serve as a diagnostic if it is influenced by stresses such as pipetting or culture in assisted reproduction technology and during in vivo stress such as poor diet.

POSTER #15.

Long-term Effects of Stress on Embryonic and Trophoblast Stem Cells is through Global Changes in Effectors of Differentiation

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Key Words: *ES cells; TS cells; Stress; mRNA; Differentiation*

Embryonic stem cells (ESC) are derived from the blastocyst and are LIF-dependent whereas placental trophoblast stem (TSC) are derived from the blastocyst and are FGF4-dependent. In TSC culture, FGF4 removal emulates normal differentiation, but we have determined that addition of hyperosmolar stress to TSC causes global changes in mRNA transcription that are dominant over FGF4 and cause differentiation. In TSC, multiple transcription factor mRNA and proteins are activated by stress including hairy enhancer

of split (HES)1, heart and mesoderm differentiation (HAND)1, eomesodermin, and retinoic response gene (STRA). These factors mediate the first differentiation the placental lineage requires in the implanting embryo. In, ESC we anticipate that factors mediating endoderm differentiation such as GATA4 and GATA6 will be upregulated on the protein and mRNA levels by hyperosmolar stress. In TSC, global mRNA data suggest a rapid response at 0.5hr of lessened macromolecular synthesis as a small number of highly changing mRNA (>1.5-fold compared with unstressed cells) and the change is by downregulation. By 24hr there is an adaptation to stress as an approximate ten-fold increase in highly changing mRNA and over half of this is by upregulation. For ESC, analysis of global mRNA change has not been performed. But, protein data suggest the same type of adaptation to stress seen in TSC. We anticipate that global mRNA assays will show similarities and differences in the kinetics of the response to stress for ESC and TSC.

POSTER #16.

Comparison of Adipogenesis in Human and Canine Mesenchymal Stem Cells

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Keywords: Mesenchymal *Stem Cells, Adipogenesis, PPARγ, Linoleic acid, Canine Model*

Understanding the process of adipogenesis is critical to the study of diseases related to energy metabolism and obesity. Mesenchymal stem cells (MSCs) offer an excellent experimental model system to explore the adipogenic process. Comparative studies provide a special window to the mechanistic basis of adipogenesis. Our lab has successfully isolated and extensively characterized MSCs from both human and dog adipose tissues. Our earlier studies have revealed the differences in the effectiveness of different induction regimens between these two species. This study was undertaken to assess the species-specific differences in terms of expression of a panel of genes important in adipogenesis: CEBP alpha, PPAR gamma, FABP4, LPL, and Leptin. Both human and canine MSCs were divided into following six experimental groups: 1) Control in high glucose DMEM + 5% FBS 2) RDI (Rosiglitazone, Dexamethasone, and Insulin) + 5% FBS 3) RDI + Linoleic acid + 5% FBS 4) RDI + 5% Rabbit Serum 5) Linoleic acid + 5% FBS and 6) IDII (Indomethacin, Dexamethasone, Insulin, and IBMX) + 5% FBS. Primers for each target gene have been designed and validated. Quantitative RT-PCR is being carried out to evaluate the pattern of gene expression across each treatment group between the two species. This study will elucidate the role of different inducing agents on gene expression and differentiation of MSCs into adipocytes.

POSTER #17.

Isolation and Characterization of Canine Kidney Epithelial Stem Cells that Differentiate into Dome-Forming Tubular Cells

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Keywords: *Kidney multipotent cell, tubular, stat-3*

Background: Dome-forming cells are considered as tubular cells in different tissues such as mammary epithelial cells and kidney epithelial cells. The isolation and characterization of normal kidney epithelial stem cells that give rise to dome-forming tubular cells have not been previously reported.**Objective:** The objective of this study is to isolate and characterize canine kidney epithelial stem cells that give rise to dome-forming tubular cells.**Methods**: Isolation of putative canine kidney epithelial stem cells---Adult canine kidney tissues were dissociated and cultured in a modified Keratinocyte SFM medium supplemented with N-acetyl-L-cysteine, L-ascorbic acid 2-phosphate, nicotinamide and fetal bovine serum. Dome-forming colonies were isolated for further characterizations. Characterizations---Dome-forming colonies were characterized for cell proliferation potential, multipotential differentiation, gap-junctional intercellular communication (GJIC) by scrape-loading dye transfer technique, anchorage-independent growth, dome-forming ability in the presence and absence of chemicals that induce or inhibit stat-3, and gene expression by RT-PCR and immunostaining. **Results:** Dome-forming kidney epithelial cell culture can be developed from adult canine kidney tissues. Besides dome cells, these cells were also capable of differentiation into osteocytes and adipocytes. These cells have been shown to grow continuously in vitro for more than 8 months and more than 30 cumulative population doublings. Similar to the immortal canine cell

line, MDCK, the dome-forming progenitor cells were deficient in GJIC, capable of anchorage-independent growth, and found to express stem cell markers, Oct-4 and Sox-2. Furthermore, sustained growth was observed only at high density cell culture (>4000/cm^2). The self-renewal of undifferentiated cells and the ability of dome formation were restricted to the high cell density area at the center of colonies. A stat-3 phosphorylation inhibitor, AG 490, inhibited the foci and dome formation. These cells on Matrigel were also capable of forming budding tubule-like organoids. **Conclusions:** Dome-forming progenitor cells with high proliferation potential and multipotent differentiation ability can be developed from adult canine kidney tissues using our cell culture techniques. These features of self-renewal and differentiation ability, in addition to the deficiency of GJIC, the expression of Oct-4 and Sox-2 stem cell markers, indicate that these cells are kidney epithelial stem cells. The density-dependent growth and the effect of stat-3 inhibitor are consistent with the hypothesis that high cell density promotes stat-3 expression, which at once promotes stem cell self-renewal and differentiation into tubular cells. These dome-forming kidney stem cells will be useful for development of an animal model for stem cell-based reparative medicine for kidney disease.

POSTER #18.

A General Mechanism for Transcription Regulation by Oct1 and Oct4 in Response to Genotoxic and Oxidative Stress

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Keywords: *Oct4, Oct1, transcription, protein phosphorylation, mass spectrometry*

Oct1 and Oct4 are homologous transcription factors with similar DNA binding specificities. Here we show that Oct1 is dynamically modified in vivo following exposure of cells to oxidative and genotoxic stress. We further show that stress regulates the selectivity of both proteins for specific DNA sequences. Mutation of two phosphorylation target residues, conserved between Oct1 and Oct4, demonstrates their role in regulating binding selectivity. Using chromatin immunoprecipitation and a native genomic site that binds four Oct1 molecules in a cooperative complex, we show that association of Oct1 with a subset of in vivo targets is inducible by stress, and that Oct1 is essential for a normal post-stress transcriptional response.

POSTER #19.

Trans-differentiation Potential of Canine Adipose-Derived Mesenchymal Stem Cells: Expression of Neural Markers at mRNA and Protein Levels

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Keywords: *Mesenchymal Stem Cells, Trans-differentiation, Neural Markers, Canine Model*

The potential of mesenchymal stem cells (MSCs) to differentiate into mesodermal cells is well established. There is growing evidence that MSCs also may trans-differentiate into cell types of other germ layer lineages. Further investigation of such phenotypic plasticity will provide important insights into their suitability for cell-based regenerative and reparative medicine. The purpose of this study was to evaluate the expression of neural markers after treatment of canine adipose derived mesenchymal stem cells (cAD-MSCs) with neural induction regimen. cAD-MSCs were isolated and expanded in culture in a low calcium medium supplemented with antioxidants. Differentiation was induced in serum free media with different induction agents. Panels of neural markers were used to evaluate the change in mRNA and protein expression by conventional and quantitative reverse transcription (RT)-PCR, and immunocytochemistry. RT-PCR data revealed that induced cells strongly expressed mRNAs for neural genes. In addition, immunocytochemistry showed that only induced cells with neural morphology, but not the undifferentiated MSCs, expressed neural markers. Thus, these findings reveal that canine MSCs can be induced to become neural-like cells under suitable conditions, which not only assume neural morphologies but also express neural markers at RNA and protein levels. This study demonstrates that MSCs from an abundant and accessible source have the potential to transdifferentiate into neural cells. While further functional studies are warranted, such cells hold great potential for treatment of neurodegenerative diseases. Evaluation of these cells in the canine system and their use in interventional studies in the canine will facilitate translational studies in the human.

POSTER #20.

Identification of Gastric Progenitor Cells

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Adult stem cells possess characteristics of self-renewal and multi-lineage differentiation. The epithelial stem cells in the stomach are responsible for constant renewal of the epithelium through generation of multiple gastric cell lineages. We identified an intestinal promoter that is active in a rare subpopulation of gastric epithelial cells and investigated whether these cells possess multilineage potential. Using a lineage tracing model, we show that these rare LacZ positive cells give rise to multilineage gastric cells. These stem cells are small in size with a high nucleus to cytoplasm ratio and are mainly detectable in the antrum of the stomach from the 15.5 dpc embryo to the adult. The entire adult stomach has approximately 300 such cells distributed as 1 per pit. After IFN□ treatment, the number of these cells increases significantly. These progenitor cells can give rise to parietal cells, enteroendocrine cells and mucous cells even though they themselves are negative for gastric cell type markers. This discovery will provide new insight into gastric stem cell biology, lineage choice and potentially, mechanisms underlying intestinal metaplasia and gastric cancer.

POSTER #21.

Adipose Tissue as a Promising Source of Mesenchymal Stem Cells: Studies in a Canine Model

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Keywords: *Mesenchymal Stem Cells, Adipogenesis, Osteogenesis, Chonrogenesis, Gene Expression, Canine Model*

Mesenchymal stem cells (MSCs) hold great promise for regenerative and reparative medicine. MSCs have been isolated from various sources including bone marrow, adipose tissue, placenta and umbilical cord blood. Dog is a promising biomedical model for evaluation of novel therapies such as those employing stem cells in experimental and in spontaneous disease settings. This study is the first documentation of isolation and characterization of mesenchymal stem cells from canine adipose tissues. The canine adipose derived mesenchymal stem cells (cAD-MSCs) were isolated from subcutaneous adipose tissues, and expanded in a low calcium medium supplemented with N-acetyl-L-cysteine and L-ascorbic acid-2-phosphate. The cAD-MSCs differed from human adipose derived mesenchymal stem cells (hAD-MSCs) in terms of proliferation potential and their responsiveness to the conventional induction regimen. The differentiation of cAD-MSCs into osteoblasts and adipocytes was effectively achieved under modified conditions, by using laminin-coated plates and PPARγ ligands, respectively. The formation of micromass was sufficient to induce chondrogenesis, unlike hAD-MSCs, which require TGF-β. These cells displayed anchorage independent growth in soft agar, and their colony forming efficiency in plastic was comparable with human counterparts. The cAD-MSCs expressed genes associated with pluripotency while their differentiated progeny expressed appropriate lineage specific genes. With the establishment of these methods, this study lays the foundation for the development of the dog as a model system for regenerative medicine as well as for probing the role of stem cells in pathologic processes such as cancer.

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The First Midwest Conference on Stem Cell Biology and Therapy

EXIT SURVEY

1. **How satisfied were you with the registration process?**
   ☐Very Dissatisfied ☐ Dissatisfied ☐ Satisfied ☐Very Satisfied

2. **How satisfied were you with the conference materials provided?**
   ☐Very Dissatisfied ☐Dissatisfied ☐Satisfied ☐Very Satisfied

3. **Overall, how satisfied were you with the speakers?**
   ☐Very Dissatisfied ☐Dissatisfied ☐Satisfied ☐Very Satisfied

4. **Overall, how satisfied were you with the conference facilities?**
   ☐Very Dissatisfied ☐ Dissatisfied ☐Satisfied ☐Very Satisfied

5. **The content of conference sessions was appropriate and informative.**
   ☐Strongly Disagree ☐Disagree ☐Agree ☐Strongly Agree

6. **The conference was well organized.**
   ☐Strongly Disagree ☐Disagree ☐Agree ☐Strongly Agree

7. **Conference staff members were helpful and courteous.**
   ☐Strongly Disagree ☐Disagree ☐ Agree ☐ Strongly Agree

8. **Would you recommend this conference to others?**
   ☐Yes ☐ No ☐Unsure

9. **Please comment on aspects you liked the most, least, and how we can improve in the future.**

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Please turn-in the exit-survey and give to a staff member at the registration table.

Thank you for your participation!