



2009

Meeting of Medical Fellows, Research Scholars,
and Physician-Scientist Early Career Awardees

Research Training Fellowships for Medical Students
HHMI-NIH Research Scholars Program
Physician-Scientist Early Career Award

Program and Abstracts
May 17–20, 2009

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Office of Grants and Special Programs

3	Introduction
4	Program Schedule
8	Keynote Speaker
9	2008 Early Career Awardees' Biographies
15	Physician-Scientist Career Panel Members' Biographies
16	Schedule of Presentations
29	Abstracts of Presentations
103	Howard Hughes Medical Institute Trustees Officers Grants and Special Programs
104	Participants
109	Index of Presentation Times
113	HHMI Conference Center Map

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Cover: Tissues of the developing heart respond to a coordinated series of extracellular signals to form the many distinct anatomical features of the mature organ. Disruption of these signaling networks contributes to congenital heart disease, the most common class of birth defects. Among these signals, vascular endothelial growth factor (VEGF) family members are critically important; however, their specific temporal roles remain incompletely defined.

Using chemical-induced expression of VEGF inhibitors in transgenic mouse embryos, we have characterized time windows during which VEGF has three distinct functions during heart development. One of these functions is to coordinate cell survival to support ventricle septation, as expression of an inhibitor of VEGF signaling (VEGFR2T) at embryonic day 11.5 (E11.5) prevents endothelialization of the ventricles and causes apoptosis specifically within the interventricular septum. These results provide a framework to understand how perturbations of VEGF signaling contribute to congenital heart defects.

The cover image shows immunofluorescent staining of the interventricular septum of a heart from an E13.5 embryo in which VEGF signaling has been blocked by the expression of VEGFR2T. Smooth muscle actin stains myocardial cells (red), PECAM stains endothelial cells (blue), and active caspase-3 stains apoptotic cells (green). Nuclei (grey) are stained with Hoechst. See abstract on page 35. (Courtesy of Gene Kew Ma, HHMI Medical Fellow, Department of Medicine, Stanford University School of Medicine. Mentor: Ching-Pin Chang, M.D., Ph.D.)

INTRODUCTION

Welcome to the 2009 Meeting of Medical Fellows, Research Scholars, and Physician-Scientist Early Career Awardees of the Howard Hughes Medical Institute. We are very pleased that participants from both HHMI medical education programs will be sharing their research and expertise in this one meeting and will be joined by the 2008 awardees of our Physician-Scientist Early Career Award program.

In 1985, HHMI launched the HHMI-NIH Research Scholars Program in partnership with the National Institutes of Health to provide outstanding students from U.S. medical schools with the opportunity to receive a year of research training at NIH. Then, in 1989, HHMI established the Research Training Fellowships for Medical Students Program to provide a similar group of students with research training in leading academic research laboratories beyond NIH. Recent years have seen the expansion of both of the programs to include dental and veterinary students, and we welcome their participation.

The Physician-Scientist Early Career Awards provide five years of research support to selected alumni of the HHMI Research Training Fellowships and HHMI-NIH Research Scholars Program as they begin their independent academic careers. The awardees will be giving oral presentations, participating in a career panel discussion on Monday evening, and co-chairing presentation sessions with the Medical Fellows and Research Scholars.

Since the inception of the research training and development programs, HHMI has supported more than 2,000 Medical Fellows and Research Scholars, and 52 Early Career Awardees. This year, 74 Medical Fellows, 49 Research Scholars, and 18 Early Career Awardees will be presenting their research. This book contains the schedule and abstracts of their presentations.

We are delighted to have Richard P. Lifton, M.D., Ph.D., as our honored speaker this year. Dr. Lifton is an HHMI investigator, chairman of the Department of Genetics, Sterling Professor of Genetics and Internal Medicine, and director of the Yale Center for Human Genetics and Genomics at Yale School of Medicine. He will discuss his laboratory's research using genetic approaches to identify the genes and pathways that contribute to common human diseases, including cardiovascular, renal, and bone disease.

We hope that the meeting will not only be a time for sharing and learning, but also a time for you to get to know your future physician-scientist colleagues better. In keeping with this objective, we have provided several informal opportunities for you to interact and network with each other.

Another way for you to continue your association with HHMI and fellow trainees is through the HHMI Alumni Network, which comprises current and former awardees. Local networks have been established in Boston, Northern California, Washington, D.C./Baltimore, Southern California, North Carolina, Chicago, Michigan, the Pacific Northwest, Texas, Cleveland, and New York City. We invite you to become involved in the HHMI alumni group nearest you and affiliate with new groups as you move about the country during your training and early career.

This meeting is held each spring so that you can present your research and exchange ideas. We have grown accustomed to high-quality work from our awardees, and this year's presentations, as judged by the abstracts, will be no exception. We congratulate you on your scientific accomplishments and development, and we want to convey our appreciation to your mentors and preceptors, whose guidance is clearly evident.

In speaking with numerous alumni of our medical education programs, we are impressed by the pivotal effect that this research opportunity has had on their career development. We hope that you will view your HHMI research experience similarly and that you will pursue further research and, ultimately, rewarding careers as physician-scientists.

Finally, we are interested in your comments and suggestions regarding both this meeting and the Medical Fellows, Research Scholars, and Physician-Scientist Early Career Award programs in general. Please direct your feedback to your respective program as follows: Medical Fellows Program to Melanie Daub at medfellows@hhmi.org; Research Scholars Program to Min Lee at research_scholars@hhmi.org; and Physician-Scientist Early Career Award to Anh-Chi Le at earlycareer@hhmi.org.

We look forward to hearing about your research and to following your careers in the years ahead.

Robert Tjian, Ph.D., *President*

Peter J. Bruns, Ph.D., *Vice President
Grants and Special Programs*

William R. Galey, Ph.D., *Director
Graduate and Medical Education Programs*

PROGRAM SCHEDULE

**2009 MEETING OF MEDICAL FELLOWS, RESEARCH SCHOLARS,
AND PHYSICIAN-SCIENTIST EARLY CAREER AWARDEES
HHMI HEADQUARTERS AND CONFERENCE CENTER, CHEVY CHASE, MARYLAND**

Sunday, May 17, 2009

5:30–6:00 p.m. **Welcoming Reception, Research Scholars and Medical Fellows, *Great Hall***

5:30–7:00 p.m. Dinner, Early Career Awardees, *Rathskeller*

6:00–7:00 p.m. Dinner, Research Scholars and Medical Fellows, *Dining Room*

7:00 p.m. **Opening Remarks, *Auditorium***
William R. Galey, Ph.D., Director, Graduate and Medical Education Programs
Howard Hughes Medical Institute

Welcoming Remarks

Peter J. Bruns, Ph.D., Vice President, Grants and Special Programs
Howard Hughes Medical Institute

Early Career Awardees Introductions

Panel Discussion: Pathway to Becoming a Physician-Scientist

Moderator:

William R. Galey, Ph.D.

Early Career Awardee Panelists:

Ari Green, M.D., University of California, San Francisco, School of Medicine

Regina LaRocque, M.D., Massachusetts General Hospital

Eduardo Méndez, M.D., University of Washington Medical Center

Mark Onaitis, M.D., Duke University School of Medicine

Rathskeller open until 10:30 p.m.

Monday, May 18, 2009

- 8:00 a.m. Breakfast, *Dining Room*
- 9:00–10:30 a.m. **Platform Presentations**
Biomedical Engineering, Biochemistry, and Bioinformatics, *Room D-124*
Molecular and Cancer Biology, *Room D-125*
Cancer Biology I, *Auditorium*
- 10:30–10:45 a.m. Break, *Great Hall*
- 10:45 a.m.–
12:30 p.m. **Platform Presentations**
Immunology and Developmental Biology I, *Room D-124*
Vascular and Cell Biology, *Room D-125*
Cancer Biology II, *Auditorium*
- 12:30 p.m. Lunch, *Dining Room and Rathskeller*
- 1:30–2:45 p.m. **Early Career Awardees' Plenary Presentations, Auditorium**
John T. Chang, M.D., University of Pennsylvania School of Medicine
Yvonne R. Chan, M.D., University of Pittsburgh School of Medicine
Costi Sifri, M.D., University of Virginia Health Sciences Center
Todd A. Fehniger, M.D., Ph.D., Washington University School of Medicine
- 2:45–3:00 p.m. Break, *Great Hall*
- 3:00–4:00 p.m. **Poster Session A, Atrium**
- 4:00–5:00 p.m. **Poster Session B, Atrium**
- 5:00–5:30 p.m. **Reception, Atrium**
- 5:30–7:00 p.m. Dinner, *Dining Room*
- 7:00 p.m. **Keynote Speaker, Auditorium**
Richard P. Lifton, M.D., Ph.D., Investigator, Howard Hughes Medical Institute; Chairman of the Department of Genetics, Sterling Professor of Genetics and Internal Medicine, Director of the Yale Center for Human Genetics and Genomics, Yale School of Medicine
- Rathskeller open until 10:30 p.m.

PROGRAM SCHEDULE

Tuesday, May 19, 2009

- 7:45 a.m. Breakfast, *Dining Room*
- 8:45–10:15 a.m. **Platform Presentations**
Infectious Disease, *Room D-124*
Cell and Developmental Biology, *Room D-125*
Stem Cell Biology, *Auditorium*
- 10:15–10:30 a.m. Break, *Great Hall*
- 10:30 a.m.–
Noon **Platform Presentations**
Immunology III, *Room D-124*
Neuroscience I, *Room D-125*
Genetics, *Auditorium*
- Noon Lunch, Research Scholars and Medical Fellows, *Dining Room*
- Noon Lunch and Workshop, Early Career Awardees, *Rathskeller*
- 1:30–2:30 p.m. **Panel Discussion: Balancing Career and Family, Auditorium**
Moderators:
Matthew Goldstein, Medical Fellow
Mari Johanna Tokita, Research Scholar
Physician-Scientist Panelists:
Donald L. Gilbert, M.D., M.S., Cincinnati Children's Hospital Medical Center
William Matsui, M.D., Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine
Christine Seroogy, M.D., University of Wisconsin–Madison School of Medicine and Public Health
Jennifer U. Sung, M.D., M.B.A., Wilmer Eye Institute, Johns Hopkins University
- 2:45 p.m. **Depart for Social/Networking Event**
- 3:00–4:30 p.m. **Social/Networking Event, National Naval Medical Center**
- 5:00 p.m. **Return to HHMI Headquarters**
Reception, Great Hall
- 5:30–6:30 p.m. Dinner, *Dining Room*
- 6:30–7:30 p.m. **Poster Session C, Atrium**
- 7:30–8:30 p.m. **Poster Session D, Atrium**
Dessert, *Atrium*
Rathskeller open until 10:30 p.m.

Wednesday, May 20, 2009

- 8:15 a.m. Breakfast, *Dining Room*
- 9:15–10:45 a.m. **Platform Presentations**
 Epidemiology and Genetics, *Room D-124*
 Neuroscience II, *Room D-125*
- 10:45 a.m. **Medical Fellows' Assembly**, *Auditorium*
- 10:45–11:00 a.m. **Research Scholars' and Early Career Awardees' Break**, *Great Hall*
- 11:00 a.m. **Recognition Ceremony**, *Auditorium*
- Opening Remarks**
 William R. Gale, Ph.D.
 Director, Graduate and Medical Education Programs
 Howard Hughes Medical Institute
- Remarks**
 Peter J. Bruns, Ph.D.
 Vice President, Grants and Special Programs
 Howard Hughes Medical Institute
- President's Remarks**
 Robert Tjian, Ph.D.
 President
 Howard Hughes Medical Institute
- Presentation of Fellows' Certificates**
- Noon Lunch, *Dining Room and Rathskeller*
- 1:30 p.m. Adjournment

Extreme Outliers as Models of Common Human Disease

RICHARD P. LIFTON, M.D., PH.D.

Investigator, Howard Hughes Medical Institute; Chairman of the Department of Genetics, Sterling Professor of Genetics and Internal Medicine, Director of the Yale Center for Human Genetics and Genomics, Yale School of Medicine

■ We have used the investigation of extreme outliers in the human population to identify genes and pathways that contribute to risk of cardiovascular, renal, and bone disease. A major focus of our work has been on hypertension, a trait that affects more than a billion people worldwide. This trait has variously been proposed to be a primary consequence of abnormalities in diverse organ systems. Our investigation of thousands of patients from around the world has identified renal salt handling as a principal determinant of long-term blood pressure homeostasis in humans. Mutations that increase net renal salt reabsorption raise blood pressure, whereas mutations that reduce salt reabsorption lower blood pressure. These mutations identify the elements that mediate and regulate the salt-handling pathway, including a novel gene family, the Wnk kinases, which we have shown regulate the balance between salt reabsorption and potassium secretion. New sequencing technologies support identification of rare variants with large effects. These findings have provided insight into both rare and common forms of blood pressure variation, have modified therapeutic approaches, and have identified new targets with the potential of having larger beneficial and fewer adverse effects.



Paul Fetters

Dr. Lifton is an HHMI investigator, Chairman of the Department of Genetics, Sterling Professor of Genetics and Internal Medicine, and Director of the Yale Center for Human Genetics and Genomics at Yale School of Medicine. He received his Ph.D. in biochemistry from Stanford University and an M.D. from Stanford University School of Medicine. Dr. Lifton completed a residency and chief residency in internal medicine at the Brigham and Women's Hospital. His laboratory has used human genetics and genomics to identify causes of heart, kidney, and bone disease. By investigating thousands of families from around the world, his group has identified more than 25 human disease genes. These include key genes and pathways that are critical to the risk of hypertension, stroke, heart attack, and osteoporosis. These studies have provided new diagnostic and therapeutic approaches to these diseases, which affect more than 1 billion people worldwide. Dr. Lifton's honors include election to the National Academy of Sciences and the Institute of Medicine, and he was awarded the 2008 Wiley Prize in Biomedical Sciences.

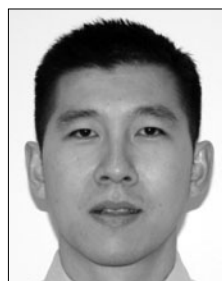
■ **Yvonne R. Chan, M.D.**, is an assistant professor of medicine in the Division of Pulmonary, Allergy, and Critical Care Medicine (PACCM) at the University of Pittsburgh. She also regularly attends on the university's Medical Intensive Care Unit and Pulmonary Transplant services. Dr. Chan participated in the HHMI Medical Fellows Program in



1997–1998 and is a recipient of the HHMI Continued Support Award. She received her M.D. from Harvard Medical School in the Health Sciences and Technology Program in 2001. She finished her clinical training in internal medicine at Mount Auburn Hospital

in Cambridge, Massachusetts, in 2004 and went on to graduate from pulmonary and critical care fellowship at the University of Pittsburgh in 2007. Concurrently, she completed postdoctoral research training in pulmonary host defense and lung immunology under the mentorship of Dr. Jay Kolls at Children's Hospital of Pittsburgh and Dr. Prabir Ray in the Division of PACCM. Dr. Chan's research focuses on innate defense against bacterial pneumonias. Most recently, she has studied lipocalin 2, an antimicrobial protein, and its mechanism of induction in bacterial infection. Her current studies involve characterization of the host signaling response to lipocalin 2. In addition, her projects include characterization of host inflammatory immune responses in chronic bacterial infection and colonization in cystic fibrosis (CF). This bedside-to-bench translational study characterizes human lung T cells, isolated from lung explants obtained from CF patients undergoing transplant. Dr. Chan's research characterizes the T cell response to chronic colonizers in CF, such as *Pseudomonas* and *Aspergillus*, in an effort to identify culprit immunological mechanisms responsible for the lung damage seen in late-stage CF.

■ **John T. Chang, M.D.**, is an instructor of medicine in the Division of Gastroenterology at the University of Pennsylvania. He obtained his B.S. degree in biological sciences from Stanford University and his M.D. from Temple University. During medical



school, he undertook research training as an HHMI-NIH Research Scholar from 1997 to 1999 in the laboratory of Dr. Ethan Shevach. He completed a residency in internal medicine and a fellowship in gastroenterology at the University of Pennsylvania. While a postdoctoral

fellow in the laboratory of Dr. Steven Reiner, he found that T cells divide asymmetrically when confronting microbial pathogens. The discovery of asymmetric T cell division was recognized as one of the journal *Science's* Top 10 Breakthroughs of 2007. Dr. Chang's research focuses on the differentiation of T lymphocytes during immune responses against microbial pathogens and during autoimmunity.

■ **Hyung J. Chun, M.D.**, is an instructor at Stanford University School of Medicine. He received his undergraduate degree in biochemical sciences from Harvard University. He subsequently received his M.D. from the Johns Hopkins University School of Medicine, during which time he participated in



the HHMI-NIH Research Scholars Program from 1999 to 2001, working in the laboratory of Dr. Michael Lenardo. His work led to the identification of a novel human mutation in the caspase-8 gene, which leads to an inherited immunodeficiency syndrome. His research

also characterized a novel role for caspase-8 in immune activation. He continued his medical training in internal medicine and cardiovascular medicine at the Stanford University School of Medicine. Dr. Chun's current research focuses on the role of G protein-coupled receptors in the vasculature. He is interested in characterization of the apelin-APJ signaling pathway, which he has recently identified to have an important role in protecting against vascular injury in rodent models of atherosclerosis and aneurysms.

■ **Todd A. Fehniger, M.D., Ph.D.**, is an assistant professor of medicine at the Washington University in St. Louis School of Medicine. He was introduced



to basic and translational research as an HHMI Medical Fellow in 1996–1997 studying natural killer cell modulation in AIDS-malignancy patients receiving low-dose interleukin-2 therapy. He received his Ph.D. in 2000 and his M.D. in 2002 from Ohio State University, where

he studied the role of cytokine-cytokine receptor signals in natural killer cell development and function. From 2002 to 2008, he completed a clinical residency in internal medicine and fellowship training in medical oncology at the Washington University in St. Louis School of Medicine. As a postdoctoral fellow from 2005 to 2008, his studies focused on the cytotoxic effector mechanisms

2008 EARLY CAREER AWARDEES' BIOGRAPHIES

utilized by lymphocytes to kill tumor and virally infected cells. Dr. Fehniger's research focuses on investigating 1) the role of microRNAs in regulating natural killer cell biology and 2) approaches to translate our basic understanding of natural killer cells into novel treatments for patients with hematologic malignancies.

■ **Matthew Freedman, M.D.**, is an assistant professor in medicine at Harvard Medical School and at the Dana-Farber Cancer Institute. He received a B.S. degree in economics and his M.D. from the University of Michigan. From 1992 to 1993, he



studied human genetics in the laboratory of Dr. Francis Collins. He then spent 1993–1994 as an HHMI-NIH Research Scholar in Dr. Michael Lenardo's laboratory. He completed internship and residency training in internal medicine at the University of Michigan

Hospital in 1996. From 1998 to 2002, he was a fellow in medical oncology in the Dana-Farber/Partners cancer care training program. From 2000 to 2005, he studied human genetics as an HHMI Physician Postdoctoral Fellow with Dr. David Altshuler at Massachusetts General Hospital and at the Whitehead genome center (later changed to the Broad Institute of Harvard and MIT). His laboratory primarily focuses on understanding the functional consequences of inheriting non-protein coding risk alleles discovered through genome-wide association studies.

■ **Timothy E. Graham, M.D.**, is an instructor in medicine and assistant professor of medicine at Harvard Medical School. He received his B.A. degree in liberal arts at St. John's College in Annapolis, Maryland, in 1990. He received additional premedical training at the University of Pennsylvania from 1991 to 1993;



during that time he learned basic biochemistry and molecular biology studying heterotrimeric G proteins in the laboratory of Dr. David R. Manning. He received his M.D. from the University of

New Mexico (UNM) School of Medicine in 1998. In 1996–1997, he was an HHMI Medical Fellow in the laboratory of Dr. Janet M. Oliver at the UNM Cancer Research and Treatment Center, where he studied the role of Ras family small GTPases in immune cell antigen receptor signaling. In 2002, he completed the Basic Scientist-

Clinician Training Program of the American Board of Internal Medicine at UNM; during this time he served as a postdoctoral research fellow under the mentorship of Dr. Richard I. Dorin. In 2003, he joined the laboratory of Dr. Barbara Kahn at Beth Israel Deaconess Medical Center, where he completed a clinical fellowship in endocrinology, diabetes, and metabolism and began work in the field of obesity, insulin resistance, and type 2 diabetes. In addition to the HHMI Early Career Award, he has received the NIDDK Clinical Scientist Development Award (K08) and related R03, the Doris Duke Charitable Foundation Clinical-Translational Scientist Award, and the Smith Family/Medical Foundation Award.

■ **Ari Green, M.D.**, is the Debbie and Andy Rachleff Distinguished Chair in Neurology, director of the Neurodiagnostics Center, assistant director of the Medical School Center, and assistant professor at the University of California, San Francisco, School of Medicine. He is a graduate of the Duke University School of Medicine and



was an HHMI Medical Fellow in 1999–2000, in the laboratory of Dr. Jorge Oksenberg. Dr. Green completed clinical training in internal medicine and neurology at the University of California, San Francisco, before serving as co-chief resident in

neurology. He had additional training in clinical neuroimmunology and neuroophthalmology under the supervision of Dr. Stephen Hauser and Dr. William Fletcher Hoyt. He was awarded the National Multiple Sclerosis Society (NMSS) and American Academy of Neurology Foundation (AANF) Career Fellowship in 2005. Dr. Green's primary research interests involve understanding the visual system in multiple sclerosis (MS) and improving methods for tracking the disease and predicting disease course. He is interested in using advanced retinal imaging and electrophysiology to investigate the retina and optic nerve as a model pathway in MS. This work is intended to help unravel the relationships between inflammation, demyelination, and neurodegeneration in the disease. Through collaboration with colleagues at Queens University Belfast, Dr. Green has advanced the understanding of retinal pathology in MS. He has continued to work on projects aimed at using retinal imaging to better investigate this pathology in MS. His laboratory work centers on using retinal imaging in conjunction with molecular methods to help improve the understanding of axon injury in MS.

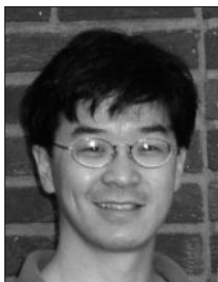
■ **Fred H. Hsieh, M.D.**, is on the faculty as a staff physician at the Cleveland Clinic and sees allergy and immunology patients, with special emphasis on patients who suffer from mastocytosis and hypereosinophilic syndromes. He received his M.D. from the Brown University School of Medicine and was an HHMI-NIH Research Scholar in 1992–



1993 with Dr. Michael M. Gottesman. He subsequently trained in internal medicine at the Johns Hopkins Hospital and in allergy and immunology at the Brigham and Women's Hospital, where he was mentored in the lab by Drs. K. Frank Austen and Joshua A. Boyce.

Dr. Hsieh's work has been supported by an NIH K08 grant and has been recognized in the past by the American Academy of Allergy, Asthma, and Immunology (AAAAI) Respiratory Diseases Research Award, the Glaxo-Wellcome Allergy Fellowship Award, and the AAAAI/Sepracor Research Excellence Award. He currently serves on the editorial board of the *Annals of Allergy, Asthma, and Immunology*; is the president of the Cleveland Allergy Society; and has been an ad hoc reviewer for several organizations, including the National Heart, Blood, and Lung Institute.

■ **Hanlee P. Ji, M.D.**, is an assistant professor in the Division of Oncology, Department of Medicine, at Stanford University School of Medicine. He is also the senior associate director of the Stanford Genome Technology Center, facilitating genome center projects geared toward clinical applications



and helping guide the center toward the application of novel technologies to clinical problems. In addition, Dr. Ji is an attending oncologist and clinical geneticist at the Stanford Cancer Center and Palo Alto Veteran's Administration Medical Center. He completed clinical training

at the University of Washington and Stanford University, and he attended the Johns Hopkins University Medical School and Reed College. He was an HHMI Medical Fellow in 1991–1992. His research is focused on characterizing the impact of combinatorial mutations and other genetic variants on cancer clinical phenotype. The specific goals of his research program are 1) developing innovative open-access strategies of deconstructing cancer genomes through sequencing, 2) identifying critical genetic events in colorectal cancer and other gastrointestinal malignancies influencing tumor be-

havior, and 3) translating those findings into prognostic and predictive genetic biomarkers that can be used clinically. To achieve these goals, his group is pioneering new approaches and the development of genomic technologies to accomplish high-throughput and high-resolution somatic mutation analysis of cancer. This effort relies on integrating novel molecular assays, developing next-generation sequencing technology, and creating bioinformatics to handle large-scale sequence data analysis. Dr. Ji also has an active translational research program addressing key questions in the management of colon cancer and others.

■ **Regina LaRocque, M.D.**, is an assistant in medicine at Massachusetts General Hospital. She received a B.S. degree in chemistry and B.A. degree in Spanish from Emory University, an M.P.H. (concentration in international health) from the Harvard School of Public Health, and an M.D.



from Duke University. She was an HHMI-NIH Research Scholar in 1995–1996 in the laboratory of Dr. Mary Ann Robinson, where she studied HLA-associated nonresponse to the hepatitis B vaccine. She completed residency training in internal medicine at

Brigham and Women's Hospital, followed by a clinical and research fellowship in infectious diseases in the combined program of Brigham and Women's Hospital and Massachusetts General Hospital. Her postdoctoral training was in the laboratory of Dr. Stephen Calderwood, where she studied host-pathogen interactions in *Vibrio cholerae* infection. In 2007, she joined the faculty of the Division of Infectious Diseases at Massachusetts General Hospital. Dr. LaRocque's current research is performed in collaboration with the International Centre for Diarrheal Disease Research in Dhaka, Bangladesh, and is focused on identifying human genetic determinants of *Vibrio cholerae* infection in an endemic setting. Her clinical work is in the area of consultative infectious diseases and travel medicine. In addition to the HHMI Early Career Award, she has received an International Research Scientist Development Award from the NIH's Fogarty International Center and a Claflin Distinguished Scholar Award from the Massachusetts General Hospital.

■ **Eduardo Méndez, M.D.**, is an assistant professor in the Department of Otolaryngology who specializes in head and neck surgical oncology and reconstruction at the University of Washington Medical Center and a molecular epidemiologist. He was an

2008 EARLY CAREER AWARDEES' BIOGRAPHIES

HHMI-NIH Research Scholar in 1996–1997. His research focuses on markers of disease progression in oral cancer. Despite advances in surgery and



chemotherapy, survival rates for oral cancer have not improved in the past two decades. Once the disease spreads in the body, survival rates drop. Dr. Méndez recently published the first study that has identified a “genetic signature” for poor survival rates in patients with oral cancer. The study also addresses how genetic signatures complement clinical information in predicting survival. He now wants to discover which genes are related specifically to the spread of oral cancer to other parts of the body. He will compare the genetics of tumors that have not spread with those that have. Dr. Méndez is interested in the genetics not only of tumor cells, but also of noncancerous cells that are near a tumor when it begins to spread. His results may one day allow physicians to predict which tumors are more likely to spread, information that will, in turn, affect treatment decisions.

■ **Goutham Narla, M.D., Ph.D.**, is an assistant professor in the Departments of Genetics and Genomic Sciences and of Medicine at Mount Sinai Hospital. He is also director of physician-scientist training for the residency program at the hospital. He is a recent graduate of the Medical Scientist



Training Program at the Mount Sinai School of Medicine, and he completed his Ph.D. training with Dr. Scott Friedman. His work involved the identification and characterization of the tumor-suppressor gene KLF6 and its role in human cancer. Dr. Narla's laboratory focuses on the identification and characterization of the genes and pathways involved in cancer metastasis. By testing the functional role of the KLF6 tumor-suppressor gene and its oncogenic splice variant KLF6-SV1, Dr. Narla has identified new signaling pathways regulated by this gene family and has provided new insight into cancer diagnosis, prognosis, and treatment. Dr. Narla was an HHMI Medical Fellow in 1999–2000 and has also won numerous awards, including the Harold Lampport Biomedical Research Prize, the Graduate Research Achievement Award, and the Humanism and Excellence in Teaching Award from the Arnold P. Gold Foundation. His research has been published in 32 peer-

reviewed publications, including *Science*, *Nature Genetics*, and the *Journal of Clinical Investigation*. His early career award continues his work on the molecular mechanisms underlying cancer metastasis.

■ **Mark Onaitis, M.D.**, is assistant professor of surgery in the Division of Cardiothoracic Surgery at



Duke University Medical Center. He attended Harvard University from 1989 to 1993, where he concentrated in government. He attended medical school at Duke University and graduated in 1997. Dr. Onaitis completed general surgery training at Duke in 2004 and finished a cardiothoracic fellowship there in 2007. He was an HHMI Medical Fellow in 1995–1996. Upon completion of training, he took his present position, in which he practices thoracic oncology at both Duke University Medical Center and the Durham VA Medical Center. He has started a laboratory effort under the mentorship of Dr. Brigid Hogan and has begun to study the role of lung epithelial stem cells in cancer.

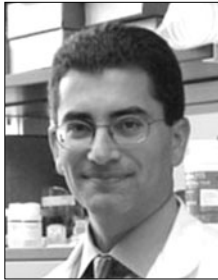
■ **Tipu S. Puri, M.D., Ph.D.**, is an assistant professor of medicine in the Section of Nephrology at The University of Chicago. He received B.A. degrees in integrated science and biochemistry, molecular biology, and cell biology from Northwestern University in 1989. He was an HHMI Medical Fellow in 1991–1992 in the laboratory of Dr. M. Marlene Hosey at Northwestern University, where he worked on the molecular cloning of the gene encoding an $\alpha 1$ subunit from human cardiac L-type calcium channels. He continued his work in Dr. Hosey's lab as part of the integrated graduate program in the life sciences and



received a Ph.D. in 1998 for his studies of the regulation of cardiac L-type calcium channel function by protein kinase-mediated phosphorylation. Dr. Puri received his M.D. from Northwestern University in 1999 and then completed residency training in internal medicine and fellowship training in nephrology at The University of Chicago. During his nephrology fellowship, he joined the lab of Dr. Richard J. Quigg, where he developed a functional murine model of chronic kidney disease (CKD) using reversible unilateral ureteral obstruction (rUUO) and identified inbred strains of mice with differential susceptibility to development of CKD

after rUUO. Dr. Puri's research focuses on determinants and mechanisms underlying susceptibility to development and progression of CKD. He is currently investigating the differences in the inflammatory responses and the process of epithelial-to-mesenchymal transition after obstruction-mediated injury in susceptible and resistant strains of mice.

■ **Benjamin Purow, M.D.**, is an assistant professor in neuro-oncology at the University of Virginia in Charlottesville. He spends about three-quarters of his time leading his laboratory and the rest is spent in



clinical care of patients with brain tumors. He received his B.A. degree in chemistry and physics, cum laude, from Harvard University in 1991 and his M.D. from Johns Hopkins Medical School in 1996. In 1994–1995, he was an HHMI Medical Fellow in the laboratory of Dr. Hyam

Levitsky at Johns Hopkins. Dr. Purow completed a residency in pediatrics at Children's National Medical Center in Washington, D.C., followed by fellowship training in pediatric hematology/oncology and in neuro-oncology at NIH. He spent several years in brain tumor research in the laboratory of Dr. Howard Fine at NIH, during which he was the first to show a role for the Notch pathway in gliomas. His research is focused on the Notch pathway and microRNAs in gliomas, with the ultimate goal of developing new therapies for these lethal cancers. In addition to the HHMI Early Career Award, he received two five-year NIH R01 awards in 2008.

■ **Joshua L. Roffman, M.D.**, is a staff psychiatrist in the Massachusetts General Hospital (MGH) Schizophrenia and Psychiatric Neuroimaging Programs and an assistant professor of psychiatry at Harvard Medical School. After studying neuroscience at Amherst



College, he attended medical school at the University of Maryland, where he participated in the Combined Accelerated Program in Psychiatry. He received additional training at the National Institute of Mental Health through the HHMI-NIH Research Scholars Program in

1998–1999 working in the laboratory of Dr. Daniel Weinberger. Following an internship in medicine at Beth Israel Deaconess Hospital and psychiatry training at MGH and McLean Hospital, he completed a postdoctoral fellowship in neuroimaging and genetics

under the mentorship of Dr. Donald Goff at MGH. Dr. Roffman's longstanding interest is in the bridging of neuroimaging and molecular markers to unravel the biological complexity of schizophrenia. He previously linked altered hippocampal-prefrontal development to reductions in neuron-specific markers, measured with in vivo magnetic resonance spectroscopy, in an animal model of schizophrenia. Currently, he is using multimodal neuroimaging and epigenetic probes to determine how common, functional variants in genes that regulate folate and dopamine metabolism contribute to abnormal patterns of prefrontal activation in schizophrenia patients. A longer term goal is to use individual variation in genetic and brain imaging markers to develop novel therapies and guide treatment selection for schizophrenia patients.

■ **Costi Sifri, M.D.**, is an assistant professor of medicine at the University of Virginia Health Sciences Center. He received his B.S. degree in biochemistry from the University of Oregon in 1989 and his M.D. from the University of Rochester in 1995. In 1992–1993, he was an HHMI-NIH Research Scholar in the Laboratory of Malaria



Research with Dr. Thomas Wellems, where he helped develop DNA transfection for the human malaria parasite *Plasmodium falciparum*. After completing residency training in internal medicine at the University of Pennsylvania, he entered clinical and research fellowships in

the Partners/Harvard Massachusetts General Hospital (MGH), Brigham and Women's Hospital, and Dana-Farber Cancer Center Combined Infectious Disease Program. In 1999, he joined the laboratories of Dr. Steve Calderwood at MGH and of National Academy of Sciences member Dr. Fred Ausubel at MGH and Harvard Medical School as an HHMI Physician Postdoctoral Fellow. There, he developed novel invertebrate model systems to characterize genetic and molecular aspects of host-pathogen interactions. These simple host-pathogen model systems allow for the simultaneous investigation of microbial pathogenesis and host innate immune responses using whole-genome approaches. Dr. Sifri's research focuses on the use of the model genetic organism *Caenorhabditis elegans* as a simple host to study host-pathogen interactions of *Staphylococcus aureus* infection. His clinical interests are in general and transplant infectious diseases. In addition to his 2007 early career award he has received an NIH Clinical Scientist K08 Development Award, the Maxwell Finland Award

2008 EARLY CAREER AWARDEES' BIOGRAPHIES

for Excellence in Infectious Disease Research from the Massachusetts Infectious Disease Society, and the Clinical Excellence Award from the Department of Medicine of the University of Virginia.

■ **Allan Tsung, M.D.**, is an assistant professor in the Department of Surgery at the University of Pittsburgh. He received his B.S. degree in biochemistry from Cornell University in 1995, and his M.D., *summa cum laude*, from SUNY Brooklyn Health Science Center in 2000. In 1997–1998, he was an HHMI-NIH Research Scholar in the laboratory of Dr. Steven A. Rosenberg at the National Cancer Institute. He completed residency training in general surgery and a hepatobiliary-pancreatic



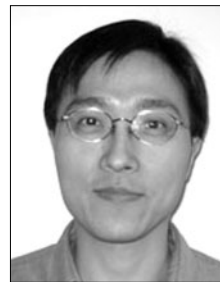
fellowship at the University of Pittsburgh Medical Center from 2000 to 2009. From 2003 to 2006, he joined the laboratory of Dr. Timothy Billiar at the University of Pittsburgh Medical Center as a research fellow studying how the innate immune system is activated during ischemic tissue injury. Dr. Tsung's research focuses on how damage to cells from a noninfectious insult, such as ischemic injury, is sensed by the body. He is studying the role of endogenous danger signals released from stressed or damaged cells in activating immune cells and subsequent inflammatory signaling. In addition to the HHMI Early Career Award, he has received an American College of Surgeons C. James Carrico Faculty Research Award and a Samuel and Emma Winters Foundation Award.

■ **Arun Venkatesan, M.D., Ph.D.**, is an assistant professor in the Department of Neurology at the Johns Hopkins University School of Medicine. He received his B.S. degree in bioengineering from the University of California, Berkeley, in 1994. He then attended medical school at the University of California, Los Angeles (UCLA), where he was an HHMI Medical Fellow in 1996–1997 in the lab of Dr. Asim Dasgupta, in the Department of Microbiology and Immunology. He completed an M.D. and Ph.D. at UCLA in 2002 (Medical Scientist Training Program; Ph.D. in microbiology and immunol-

ogy), and he completed his residency in neurology at Johns Hopkins Hospital in 2006. He then undertook a fellowship in the Richard T. Johnson Division of Neuroimmunology and Neuroinfectious Diseases at Johns Hopkins, where he joined the lab of Dr. Avindra Nath to study the effects of HIV infection and drug abuse on hippocampal neurogenesis. Dr. Venkatesan's current research, focusing on neuroinflammatory and neuroinfectious diseases, seeks to 1) delineate mechanisms by which new neurons can replace damaged cells within the brain and spinal cord and 2) determine how to protect axons, which are the "cables" that connect neurons to each other, from inflammatory or infectious damage. In addition to the HHMI Early Career Award, he has received an NIH Clinical Scientist Development Award (KO8).



■ **Paul B. Yu, M.D., Ph.D.**, is an assistant professor of medicine in the Cardiology Division at Massachusetts General Hospital (MGH). He completed an A.B. degree in philosophy and a B.S. degree in biological sciences at Stanford University, followed by an M.D., and a Ph.D. in immunology, at Duke University, studying innate humoral immune barriers to xenotransplantation. He trained in internal medicine at the University of California, San Francisco, and completed a clinical fellowship in cardiology at MGH. Following clinical training, Dr. Yu pursued postdoctoral training in the Cardiovascular Research Center at MGH in the laboratory of Dr. Ken Bloch. He was an HHMI Medical Fellow in 1995–1996. Dr. Yu's laboratory studies the pathobiology of idiopathic pulmonary arterial hypertension and other disorders involving abnormalities of the bone morphogenetic protein signaling pathway. Dr. Yu is board certified in internal medicine and cardiovascular medicine, and he practices cardiology in the Heart Center at MGH.



14 2009 Meeting of Medical Fellows, Research Scholars, and Physician-Scientist Early Career Awardees

PHYSICIAN-SCIENTIST CAREER PANEL MEMBERS' BIOGRAPHIES

■ **Donald L. Gilbert, M.D., M.S.**, is an associate professor of pediatrics and neurology; director of the Movement Disorders and Tourette Syndrome Clinics; and director of the Transcranial Magnetic Stimulation (TMS) Laboratory at Cincinnati Children's Hospital Medical Center. He received a



B.A. degree in philosophy from Princeton University, and an M.D. and M.S. degree in clinical research design and statistical analysis, both from the University of Michigan. He was an HHMI-NIH Research Scholar in 1991-1992. Dr. Gilbert trained in pediatrics

and neurology at Johns Hopkins University and is Board Certified in Neurology, with Special Qualification in Child Neurology. His diverse research interests include Tourette syndrome and ADHD genetics and physiology. His neurophysiology research has involved motor cortex inhibitory function, assessed with TMS. His TMS laboratory also studies neuroplasticity and is embarking on treatment studies. Dr. Gilbert is an active mentor in clinical research and a preceptor for residents, and he serves on the medical advisory board for the National Tourette Syndrome Association.

■ **William Matsui, M.D.**, is an associate professor of oncology at the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine. He received his undergraduate degree in biochemistry from Harvard College and his M.D. from the University of California, San Francisco. He was an HHMI Medical Fellow in 1992-1993.



He completed his internship and residency in internal medicine at the University of Washington in Seattle and fellowship training in medical oncology at Johns Hopkins University. Dr. Matsui clinically specializes in caring for adults with hematologic malignancies

and in bone marrow transplantation. His current research focuses on studying cancer stem cells in several cancers, and he has studied the role of developmental signaling pathways in regulating both normal hematopoiesis and myeloid leukemias. His lab is also focused on translational research and has been successful in developing novel therapies that target cancer stem cells in hematologic malignancies. More than a dozen clinical trials have been initiated based on his lab's preclinical work.

■ **Christine Seroogy, M.D.**, is an assistant professor in the Department of Pediatrics and an assistant director of the Clinical and Translational Research Core at the University of Wisconsin-Madison School of Medicine and Public Health. She received her M.D. from the University of Minnesota. She was an HHMI Medical Fellow in 1991-1992. She completed her residency in pediatrics at Boston Children's Hospital and an allergy/immunology fellowship at the University of California, San Francisco. She was also a clinical instructor and a postdoctoral fellow in the laboratory of Dr. Garry Fathman at Stanford University. Dr. Seroogy's teaching responsibilities involve medical and graduate students, fellows, and pediatric physicians-in-training. She is involved in the educational curriculum for the allergy immunology fellowship, and she actively mentors several allergy/immunology fellows. Her



research interest is in the biology of regulatory T cells, and she is investigating the role of these cells in varied immunologic contexts with an emphasis on allergic inflammation.

■ **Jennifer U. Sung, M.D., M.B.A.**, is an assistant professor of ophthalmology in the Retina Division of the Wilmer Eye Institute at Johns Hopkins University. She received an M.D. from the Northwestern University Medical School and completed an internship in internal medicine at Northwestern University/Evanston Hospital. She completed ophthalmology training at the Bascom Palmer Eye Institute, a vitreoretinal fellowship at the Wills Eye Hospital, and a medical retina research fellowship at Moorfields Eye



Hospital in London. She was an HHMI-NIH Research Scholar in 1992-1993. Dr. Sung's clinical focus is on diseases of the retina and vitreous, including macular degeneration, retinal detachments, diabetic retinopathy, and macular puckers and holes.

She is actively involved in teaching medical students, ophthalmology residents, and retina fellows. Dr. Sung's research focus is in understanding neuroprotection of the retina, with the aim of translating her laboratory findings into improved prevention and treatment of retinal degenerations.

SCHEDULE OF PRESENTATIONS

MONDAY
ROOM D-124

Biomedical Engineering, Biochemistry, and Bioinformatics

page 30

Session Co-Chairs: Hanlee P. Ji and Allan S. Mabardy

- 9:00 a.m.** Prognostic and predictive genetics of colorectal cancer via cancer genome sequence deconstruction
Hanlee P. Ji, M.D., Early Career Awardee, Stanford University School of Medicine
- 9:15 a.m.** Creation of custom microarrays for identifying novel gene fusions in human malignancies
Craig P. Giacomini, Medical Fellow, Stanford University School of Medicine (Jonathan R. Pollack, M.D., Ph.D.)
- 9:30 a.m.** Changes in diffusion of macromolecules in human blood clots resulting from exposure to high-intensity focused ultrasound
Guy C. Jones, Research Scholar, University of Medicine and Dentistry of New Jersey–New Jersey Medical School (Bradford J. Wood, M.D.)
- 9:45 a.m.** Can dynamic contrast-enhanced multidetector computed tomography accurately measure fluid flow velocity?
Lisa L. Chu, Medical Fellow, University of California, San Francisco, School of Medicine (Benjamin M. Yeh, M.D.)
- 10:00 a.m.** Insulin-sensitive fusion of vesicles containing glucose transporter with the plasma membrane can be detected and characterized by total internal reflection fluorescence microscopy
Allan S. Mabardy, Medical Fellow, University of Massachusetts Medical School (Michael Czech, Ph.D.)
- 10:15 a.m.** Expansion of the rat inner medullary collecting duct phosphoproteome and quantitative mass spectrometry of urea transporter phosphorylation in response to vasopressin
Amar D. Bansal, Research Scholar, New York University School of Medicine (Mark A. Knepper, M.D., Ph.D.)
- 10:30–** Break
- 10:45 a.m.**

Immunology and Developmental Biology I

page 33

Session Co-Chairs: Allan Tsung and Gene Kew Ma

- 10:45 a.m.** Sensing danger within: role of an endogenous alarm molecule in mediating inflammation of the liver
Allan Tsung, M.D., Early Career Awardee, University of Pittsburgh School of Medicine
- 11:00 a.m.** Zymosan-mediated inflammation impairs in vivo reverse cholesterol transport in mice
Priya Malik, Medical Fellow, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Jonathan D. Smith, Ph.D.)
- 11:15 a.m.** Molecular mechanism of β -amyloid inhibition of nitric oxide signaling
Hubert Shih, Research Scholar, David Geffen School of Medicine at UCLA (David D. Roberts, Ph.D.)
- 11:30 a.m.** Immune surveillance is mediated by the antiangiogenic activity of thrombospondin-1
Lior Braunstein, Medical Fellow, Harvard Medical School (Sandra W. Ryeom, Ph.D.)
- 11:45 a.m.** LFA-1-mediated, HuR-dependent stabilization of VEGF mRNA in macrophages
Yasha Modi, Medical Fellow, Yale School of Medicine (Jeffrey R. Bender, M.D.)
- Noon** Vascular endothelial growth factor has distinct roles during heart development
Gene Kew Ma, Medical Fellow, Stanford University School of Medicine (Ching-Pin Chang, M.D., Ph.D.)

Molecular and Cancer Biology

page 36

Session Co-Chairs: Timothy E. Graham and Lucy Le He

- 9:00 a.m.** RBP-R2: a potential RBP4 receptor and retinol transporter in liver, adipose tissue, and gut
Timothy E. Graham, M.D., Early Career Awardee, Beth Israel Deaconess Medical Center
- 9:15 a.m.** An oncogenic splice variant of the Kruppel-like factor 6 (KLF6) tumor-suppressor gene promotes prostate cancer progression and metastasis
Goutham Narla, M.D., Ph.D., Early Career Awardee, Mount Sinai School of Medicine
- 9:30 a.m.** HIF-2 α in acute and malignancy-associated inflammation
Emily P. Williams, Medical Fellow, University of Pennsylvania School of Medicine (M. Celeste Simon, Ph.D.)
- 9:45 a.m.** Leflunomide activates the Notch pathway, leads to carcinoid cancer cell cycle arrest, and represents a novel potential therapeutic option
MacKenzie R. Cook, Medical Fellow, Duke University School of Medicine (Herbert Chen, M.D.)
- 10:00 a.m.** High-throughput screening identification of an inducer of 15-hydroxyprostaglandin dehydrogenase, a suppressor of human colon cancer
Lucy Le He, Medical Fellow, Case Western Reserve University School of Medicine (Sanford D. Markowitz, M.D., Ph.D.)
- 10:15 a.m.** Targeting the MET tyrosine kinase receptor to inhibit osteosarcoma metastasis
Lillian M. Guenther, Research Scholar, State University of New York Downstate Medical Center College of Medicine (Chand Khanna, D.V.M., Ph.D.)
- 10:30–10:45 a.m.** Break

Vascular and Cell Biology

page 39

Session Co-Chairs: Hyung J. Chun and Justin Poling

- 10:45 a.m.** Role of apelin-APJ signaling in the vasculature
Hyung J. Chun, M.D., Early Career Awardee, Stanford University School of Medicine
- 11:00 a.m.** Pullulan-deferoxamine delivery film for targeted ischemic preconditioning
Michael G. Galvez, Medical Fellow, Stanford University School of Medicine (Geoffrey C. Gurtner, M.D., and Amato J. Giaccia, Ph.D.)
- 11:15 a.m.** A novel IL-10 signaling pathway in vascular smooth muscle cells modulates the acute p21^{cip1}-mediated arterial wound response after vascular injury
Angela Catherine Lee, Research Scholar, Harvard Medical School (Manfred Boehm, M.D.)
- 11:30 a.m.** In situ regulation of choroidal blood flow by smooth muscle cells and pericytes: an ex vivo confocal time-lapse imaging approach in sclerochoroidal explants
Audree B. Condren, Research Scholar, University of Oklahoma College of Medicine (Emily Y. Chew, M.D., and Wai T. Wong, M.D., Ph.D.)
- 11:45 a.m.** Understanding energy production through the cell cycle: a synchronous yeast model system
Matthew J. Reilley, Research Scholar, The Warren Alpert Medical School of Brown University (Robert S. Balaban, Ph.D.)
- Noon** The *Gne*^{M712T/M71T} hereditary inclusion body myopathy mouse model displays multiple glycocalyx alterations as part of a unique glomerulopathy
Justin Poling, Research Scholar, Vanderbilt University School of Medicine (William A. Gahl, M.D., Ph.D., and Marjan Huizing, Ph.D.)
- 12:15 p.m.** Role of galectin-3 on the development of pulmonary fibrosis in Hermansky-Pudlak syndrome type 1
Caroline Yeager, Research Scholar, Duke University School of Medicine (William A. Gahl, M.D., Ph.D., and Bernadette R. Gochuico, M.D.)

SCHEDULE OF PRESENTATIONS

MONDAY
AUDITORIUM

Cancer Biology I

page 43

Session Co-Chairs: Mark Onaitis and Kristopher Bosse

- 9:00 a.m.** Analysis of the cell of origin of lung adenocarcinoma
Mark Onaitis, M.D., Early Career Awardee, Duke University School of Medicine
- 9:15 a.m.** Toward the functional validation of *BRCA1-Associated RING Domain 1* as a neuroblastoma predisposition gene
Kristopher Bosse, Medical Fellow, University of Pennsylvania School of Medicine (John M. Maris, M.D.)
- 9:30 a.m.** γ -Interferon-mediated superinduction of B7-H1 in PTEN-deficient glioma patients: an immunoresistant phenotype that can confound response to cancer vaccine therapy
Seunggu J. Han, Medical Fellow, University of California, San Francisco, School of Medicine (Andrew T. Parsa, M.D., Ph.D.)
- 9:45 a.m.** Targeting the 26S proteasome for radiotherapeutic benefit in glioblastoma multiforme
Zachary Zumsteg, Medical Fellow, David Geffen School of Medicine at UCLA (William McBride, D.Sc.)
- 10:00 a.m.** The role of cell cycle in epidermal growth factor receptor-mediated radiosensitization
Susan M. Hiniker, Medical Fellow, University of Michigan Medical School (Theodore S. Lawrence, M.D., Ph.D.)
- 10:15 a.m.** Effect of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) inhibition on tumor oxygenation, interstitial fluid pressure, and liposome delivery
Tina D. Taylor, Medical Fellow, Duke University School of Medicine (Mark W. Dewhirst, D.V.M., Ph.D.)
- 10:30–10:45 a.m.** Break

Cancer Biology II

page 46

Session Co-Chairs: Benjamin Purow and Mark P. Chao

- 10:45 a.m.** MicroRNA-7 is a potential tumor suppressor inhibiting oncogenic pathways in gliomas
Benjamin Purow, M.D., Early Career Awardee, University of Virginia School of Medicine
- 11:00 a.m.** Adipose-derived mesenchymal stem cells represent a novel delivery vehicle for therapeutic agents in the treatment of intracranial gliomas
Hasan A. Zaidi, Medical Fellow, Johns Hopkins University School of Medicine (Alfredo Quiñones-Hinojosa, M.D.)
- 11:15 a.m.** The effects of PDGFR- α stimulation on neural and brain tumor stem cell behavior and molecular signaling
Thomas Adam Kosztowski, Medical Fellow, Johns Hopkins University School of Medicine (Alfredo Quiñones-Hinojosa, M.D., and Hongjun Song, Ph.D.)
- 11:30 a.m.** Investigating the therapeutic value of Wnt/ β -catenin activation in malignant melanoma
Corinne Taraska, Medical Fellow, University of Washington School of Medicine (Andy J. Chien, M.D., Ph.D., and Randall T. Moon, Ph.D.)
- 11:45 a.m.** Engineering a tumor-specific, apoptosis-resistant T cell for adoptive cell transfer therapy
Anusha Kalbasi, Research Scholar, David Geffen School of Medicine at UCLA (Steven A. Rosenberg, M.D., Ph.D.)
- Noon** Anticancer CD4 memory T cells: identification by CD44 and CD137
Matthew J. Goldstein, Medical Fellow, Stanford University School of Medicine (Ron Levy, M.D.)
- 12:15 p.m.** CD47 is an independent prognostic factor and therapeutic antibody target on acute myeloid leukemia stem cells
Mark P. Chao, Medical Fellow, Stanford University School of Medicine (Irving L. Weissman, M.D.)

Plenary Session

page 50

- 1:30 p.m.** Different by destruction: unequal inheritance of the transcription factor T-bet as a mechanism to diversify daughter T cell fates
John T. Chang, M.D., Early Career Awardee, University of Pennsylvania School of Medicine
- 1:45 p.m.** Lipocalin 2 is required for pulmonary host defense against *Klebsiella* infection
Yvonne R. Chan, M.D., Early Career Awardee, University of Pittsburgh School of Medicine
- 2:00 p.m.** Functional genomic analysis of *Caenorhabditis elegans* innate immunity
Costi Sifri, M.D., Early Career Awardee, University of Virginia Health Sciences Center
- 2:15 p.m.** Natural killer cell microRNA transcriptome defined by massively parallel sequencing
Todd A. Fehniger, M.D., Ph.D., Early Career Awardee, Washington University School of Medicine

SCHEDULE OF PRESENTATIONS

MONDAY
ATRIUM

Poster Session A, 3:00–4:00 p.m.

page 52

Session Co-Chairs: Lauren Frazer and Jessica M. Valdez

- Poster A1** A mineralocorticoid receptor *trans*-repression pathway suppresses endothelial inflammation
Kevin P. Blaine, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Robert L. Danner, M.D.)
- Poster A2** Extracellular regulated kinase 1 attenuates stem cell factor signaling in c-Kit-dependent bone marrow cells
Karl William Staser, Medical Fellow, Indiana University School of Medicine (D. Wade Clapp, M.D.)
- Poster A3** Chlamydia-induced Toll-like receptor 2 signaling leads to increased neutrophil activation and delayed spontaneous apoptosis
Lauren Frazer, Medical Fellow, University of Pittsburgh School of Medicine (Toni Darville, M.D.)
- Poster A4** A mouse model of hyper-IgE syndrome (HIES)
Scott Steward-Tharp, Research Scholar, University of Iowa College of Dentistry, Oxford University (John J. O'Shea, M.D.)
- Poster A5** Role of chemerin, chemokine-like receptor 1, and natural killer cells in particle-mediated joint inflammation
Ryan Steven Huss, Medical Fellow, Stanford University School of Medicine (Eugene C. Butcher, M.D., and Brian A. Zabel, Ph.D.)
- Poster A6** Novel approach to genomic profiling in Sézary syndrome
Rebecca G. Pomerantz, Medical Fellow, University of Pittsburgh School of Medicine (Louis D. Falo Jr., M.D., Ph.D., and Larisa J. Geskin, M.D.)
- Poster A7** Effects of CD4 T cell precursor frequency on allospecific memory B cell differentiation
J. Brett Mendel, Medical Fellow, Emory University School of Medicine (Christian P. Larsen, M.D., Ph.D.)
- Poster A8** HIV-specific T cell responses for select antigens in exposed, uninfected men who have sex with men
Rex G. Cheng, Medical Fellow, Duke University School of Medicine (Douglas F. Nixon, M.D., Ph.D.)
- Poster A9** Loss of HIV-specific memory B cell response following initiation of antiretroviral therapy
Jenny Chen, Research Scholar, Indiana University School of Medicine (Anthony S. Fauci, M.D., and Susan Moir, Ph.D.)
- Poster A10** Influenza pandemic evolution: the role of the viral polymerase protein PB1
Brett Jagger, Research Scholar, Indiana University School of Medicine (Jeffery K. Taubenberger, M.D., Ph.D.)
- Poster A11** Investigation of cellular transactivator stimulatory protein-1 on the varicella-zoster virus open reading frame 63 promoter region
Makeda L. Robinson, Medical Fellow, Stanford University School of Medicine (Ann M. Arvin, M.D.)
- Poster A12** Magnetic resonance imaging in the detection and therapeutic monitoring of hematogenous *Candida* meningoencephalitis
Jessica M. Valdez, Research Scholar, University of New Mexico School of Medicine (Thomas J. Walsh, M.D.)
- Poster A13** Effect of oxathiazolones and their derivatives on survival of *Plasmodium falciparum*
Shaka J.D. Bahadu, Medical Fellow, Weill Cornell Medical College (Carl Nathan, M.D.)

Poster Session B, 4:00–5:00 p.m.

page 59

Session Co-Chairs: Karim Y. Helmy and Patrick W. Blake

- Poster B1** Survival of the perivascular niche following irradiation reveals heterogeneity of the radiation response in glioblastoma multiforme in vivo
Karim Y. Helmy, Medical Fellow, University of Medicine and Dentistry of New Jersey–New Jersey Medical School (Eric C. Holland, M.D., Ph.D.)
- Poster B2** Radiosensitization of glioblastoma multiforme by modulation of Met signaling with human anti-hepatocyte growth factor monoclonal antibody
Ian M. Buchanan, Research Scholar, The Warren Alpert Medical School of Brown University (Kevin Camphausen, M.D.)
- Poster B3** Relevance of Polycomb group-mediated epigenetic modifications in glioblastoma multiforme pathophysiology
Chiba Ene, Research Scholar, Indiana University School of Medicine (Howard A. Fine, M.D.)
- Poster B4** Vascular endothelial growth factor C (VEGF-C) is important in the development and metastasis of head and neck squamous cell carcinoma
Rachel L. Chard, Research Scholar, Oregon Health and Science University School of Medicine (J. Silvio Gutkind, Ph.D.)
- Poster B5** TGF- β receptor III is downregulated by Δ Np63 and methylation and contributes to altered TGF- β and NF- κ B signaling and the malignant phenotype in head and neck squamous cell cancer
Frederick Wang, Research Scholar, Yale School of Medicine (Carter Van Waes, M.D., Ph.D.)
- Poster B6** Genetic analyses of constitutive NF- κ B activation in diffuse large B cell lymphoma
Paul B. Romesser, Research Scholar, Boston University School of Medicine (Louis Staudt, M.D., Ph.D.)
- Poster B7** Induction of functionally competent interleukin-21 receptor in B cells from chronic lymphocytic leukemia patients
Rebekah Browning, Medical Fellow, The Ohio State University College of Medicine (John C. Byrd, M.D.)
- Poster B8** Mutated BCR-ABL as immunologic targets in chronic myelogenous leukemia (CML)
Ann Cai, Medical Fellow, Harvard-MIT Division of Health Sciences and Technology (Catherine J. Wu, M.D., and Jerome Ritz, M.D.)
- Poster B9** Low-fat diet reduces the progression of established bone metastases in mice
Timothy Van Johnson, Medical Fellow, Emory University School of Medicine (Leland W.K. Chung, Ph.D., and Viraj A. Master, M.D., Ph.D.)
- Poster B10** MicroRNA expression profiles associated with cancer-specific mortality in colon adenocarcinoma
Jason E. Hawkes, Research Scholar, University of Utah School of Medicine (Curtis C. Harris, M.D.)
- Poster B11** Development of mouse tumor models for multidrug resistance
Michelle Samuel, Research Scholar, University of Pennsylvania School of Veterinary Medicine (Michael Gottesman, M.D.)
- Poster B12** Clinical and molecular genetics investigations of Brooke-Spiegler syndrome and molecular modeling of CYLD mutations
Patrick W. Blake, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Jorge R. Toro, M.D.)
- Poster B13** Induction of a mesenchymal phenotype in tumor cells and a cytokine signature associated with tumor progression
Marianne D. Castillo, Research Scholar, University of Medicine and Dentistry of New Jersey–New Jersey Medical School (Jeffrey Schlom, Ph.D., and Claudia Palena, Ph.D.)
- Poster B14** Exploring mechanisms in epigenetic regulation of epidermal homeostasis
Jennifer K. Chen, Medical Fellow, Johns Hopkins University School of Medicine (Paul A. Khavari, M.D., Ph.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
ROOM D-124

Infectious Disease

page 66

Session Co-Chairs: Yvonne R. Chan and Steven Beaudry

- 8:45 a.m.** Clonal outbreak of the apicomplexan parasite *Sarcocystis neurona* in southern sea otters highlights key concepts in eukaryotic disease pathogenesis
Jered M. Wendte, Research Scholar, Oklahoma State University Center for Veterinary Health Sciences (Michael E. Grigg, Ph.D.)
- 9:00 a.m.** Role of membrane oxidation on the functional display of *Plasmodium falciparum* erythrocyte membrane protein-1, the principal virulence factor on the surface of parasitized erythrocytes
Steven Beaudry, Research Scholar, West Virginia School of Osteopathic Medicine (Rick M. Fairhurst, M.D., Ph.D.)
- 9:15 a.m.** Toward a paratransgenic approach against visceral leishmaniasis
Heidi Hillesland, Medical Fellow, University of New Mexico School of Medicine (Ravi Durvasula, M.D., and Ivy Hurwitz, Ph.D.)
- 9:30 a.m.** HIV-1 *vpr* causes DNA damage in human proximal tubule cells: insights into the mechanism of HIV-associated nephropathy (HIVAN) pathogenesis
Justin Chan, Medical Fellow, Mount Sinai School of Medicine (Paul E. Klotman, M.D.)
- 9:45 a.m.** The C proteins of human parainfluenza virus type 1 (HPIV1) suppress host innate immunity and apoptosis by blocking IRF and NF- κ B signaling
Jim B. Boonyaratankornkit, Research Scholar, University of California, San Francisco, School of Medicine (Brian R. Murphy, M.D., and Peter L. Collins, Ph.D.)
- 10:00 a.m.** Analysis of respiratory syncytial virus epitope-specific CD8⁺ T cell receptor clonotypes
Alex Ryder, Research Scholar, Vanderbilt University School of Medicine (Barney S. Graham, M.D., Ph.D.)
- 10:15–** Break
10:30 a.m.

Immunology III

page 69

Session Co-Chairs: Tipu S. Puri and Elise M. Meoli

- 10:30 a.m.** Investigation of determinants of susceptibility or resistance to development and progression of chronic kidney disease in inbred murine strains
Tipu S. Puri, M.D., Ph.D., Early Career Awardee, The University of Chicago Pritzker School of Medicine
- 10:45 a.m.** Subverting regulation: a murine model of human food allergy
Suejy Hobson, Medical Fellow, David Geffen School of Medicine at UCLA (Talal A. Chatila, M.D.)
- 11:00 a.m.** Th1 effector cells convert into regulatory T cells after encountering self-antigen in vivo
David Christopher Caretto, Medical Fellow, University of California, San Francisco, School of Medicine (Abul K. Abbas, M.D.)
- 11:15 a.m.** Transforming growth factor- β signaling abnormalities in central nervous system demyelinating diseases: differential SMAD7 expression in peripheral blood mononuclear cells from healthy donors and multiple sclerosis patients
Elise M. Meoli, Research Scholar, University of Rochester School of Medicine and Dentistry (Steven Jacobson, Ph.D.)
- 11:30 a.m.** Tolerogenicity of skin grafts in grafted tolerant animals
Joshua I. Weiner, Medical Fellow, Yale School of Medicine (David H. Sachs, M.D.)
- 11:45 a.m.** Functional and genetic characterization of a novel human immune disorder
Jeremiah C. Davis, Research Scholar, George Washington University School of Medicine and Health Sciences (Helen Su, M.D., Ph.D.)

Cell and Developmental Biology

page 72

Session Co-Chairs: Paul B. Yu and Joshua A. Gordon

- 8:45 a.m.** BMP type 1 receptor inhibition reduces heterotopic ossification
Paul B. Yu, M.D., Ph.D., Early Career Awardee, Harvard Medical School
- 9:00 a.m.** Rescuing craniofacial development in an avian model of holoprosencephaly
H. Jonathan Chong, Medical Fellow, University of California, San Francisco, School of Medicine (Ralph S. Marcucio, Ph.D.)
- 9:15 a.m.** Interaction between Wnt and BMP signaling in joint development
Joshua A. Gordon, Research Scholar, David Geffen School of Medicine at UCLA (Yingzi Yang, Ph.D.)
- 9:30 a.m.** A novel role for TRPV4 as a modulator of interleukin-1 effects on chondrocytes
Mohamad Halawi, Medical Fellow, Duke University School of Medicine (Farshid Guilak, Ph.D., and Wolfgang Liedtke, M.D., Ph.D.)
- 9:45 a.m.** Novel inflammatory gene expression and cytokine response in the MRL/MpJ mouse following a closed knee fracture
John Strudwick Lewis Jr., Medical Fellow, Duke University School of Medicine (Steven A. Olson, M.D.)
- 10:00 a.m.** Activation of liver X receptor promotes vascular cell calcification
Jeffrey J. Hsu, Medical Fellow, David Geffen School of Medicine at UCLA (Linda L. Demer, M.D., Ph.D., and Peter Tontonoz, M.D., Ph.D.)
- 10:15-** Break
- 10:30 a.m.**

Neuroscience I

page 75

Session Co-Chairs: Ari Greene and Steve Khachi

- 10:30 a.m.** Transcriptional profile of genes involved in axonal transport correlated to UHR-SD OCT from a mouse model of optic neuritis
Ari Green, M.D., Early Career Awardee, University of California, San Francisco, School of Medicine
- 10:45 a.m.** Contributions of presynaptic calcium dynamics at functionally divergent release sites to target-cell-dependent plasticity within the auditory nerve-cochlear nucleus circuit
Steve Khachi, Research Scholar, Des Moines University, College of Osteopathic Medicine (Stephan D. Brenowitz, Ph.D.)
- 11:00 a.m.** Reversal of the serotonin “reuptake” transporter
Sean McEvoy, Medical Fellow, Yale School of Medicine (George Richerson, M.D., Ph.D.)
- 11:15 a.m.** Mechanisms of seizure suppression by low-frequency electrical stimulation of the fimbria-fornix-hippocampal commissures in combined hippocampus-entorhinal cortex slices in rats
Sheela Toprani, Medical Fellow, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Dominique Durand, Ph.D., and Imad Najm, M.D.)
- 11:30 a.m.** Testing connections between steroidogenesis and neurodegeneration in Niemann-Pick disease type C
Jennifer Hong, Medical Fellow, Stanford University School of Medicine (Matthew P. Scott, Ph.D.)
- 11:45 a.m.** Characterizing blood-brain barrier disruption through fluid attenuated inversion recovery (FLAIR) imaging with matrix metalloproteinase levels in an animal model of middle cerebral artery occlusion
Ayush Batra, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Steven Warach, M.D., Ph.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
AUDITORIUM

Stem Cell Biology

page 78

Session Co-Chairs: Fred H. Hsieh and Cedar J. Fowler

- 8:45 a.m.** Effector cell development and lineage commitment in allergic inflammation
Fred H. Hsieh, M.D., Early Career Awardee, Cleveland Clinic Foundation
- 9:00 a.m.** Niche recycling through division-independent egress of hematopoietic stem cells
Agnieszka Czechowicz, Medical Fellow, Stanford University School of Medicine (Irving L. Weissman, M.D., and Deepa Bhattacharya, Ph.D.)
- 9:15 a.m.** Assessing plasticity: the populations responsible for epithelial engraftment of marrow-derived cells
Justin Brent Cohen, Medical Fellow, Yale School of Medicine (Diane S. Krause, M.D., Ph.D.)
- 9:30 a.m.** Sunitinib facilitates bone marrow engraftment through c-kit
Natasha Fewkes, Research Scholar, Oregon Health and Science University School of Medicine (Crystal L. Mackall, M.D.)
- 9:45 a.m.** Lentiviral vectors with leukocyte integrin CD11b promoter leads to efficient transduction of canine leukocyte adhesion deficiency CD34+ cells
Cedar J. Fowler, Research Scholar, Tufts University School of Medicine (Dennis Hickstein, M.D.)
- 10:00 a.m.** The immunogenic properties of human embryonic stem cells
Jeremy Pearl, Medical Fellow, Stanford University School of Medicine (Mark M. Davis, Ph.D., and Joseph C. Wu, M.D., Ph.D.)
- 10:15-10:30 a.m.** Break

Genetics

page 81

Session Co-Chairs: Eduardo Méndez and Amelia Keaton

- 10:30 a.m.** Association of genome-wide DNA copy number aberrations and gene expression in metastatic oral squamous cell carcinoma (OSCC)
Eduardo Méndez, Early Career Awardee, University of Washington Medical Center
- 10:45 a.m.** The search for endogenous regulators of transient receptor potential vanilloid 1
Asaff Harel, Medical Fellow, University of Pittsburgh School of Medicine (Joseph C. Glorioso, Ph.D.)
- 11:00 a.m.** Genotype-phenotype correlations in patients with holoprosencephaly and alterations in *TG-Interacting Factor*
Amelia Keaton, Research Scholar, University of South Carolina School of Medicine (Maximilian Muenke, M.D.)
- 11:15 a.m.** Quantitative trait loci analysis of nonalcoholic steatohepatitis
Sarina Pasricha, Medical Fellow, Northwestern University, The Feinberg School of Medicine (Richard M. Green, M.D.)
- 11:30 a.m.** Structural shifts in heat shock RNA-1: a mechanism for thermosensing
Patrick Varley, Medical Fellow, New York University School of Medicine (Evgeny Nudler, Ph.D.)
- 11:45 a.m.** Whole-genome association study and fine mapping of a locus for malignant histiocytosis in the Bernese mountain dog
Abigail Shearin, Research Scholar, University of Pennsylvania School of Veterinary Medicine (Elaine Ostrander, Ph.D.)

Poster Session C, 6:30–7:30 p.m.

page 84

Session Co-Chairs: David Henry Perlmutter and Shila Azodi

- Poster C1** Examination of the effects of inhibiting TrkB signaling on limbic epileptogenesis in Kv1.1 null mutant mice
Sima Yazdani, Medical Fellow, Duke University School of Medicine (James McNamara, M.D.)
- Poster C2** Tuberous sclerosis complex signaling regulates EphA-mediated axon guidance
Hasani K. Baharanyi, Medical Fellow, Yale School of Medicine (Mustafa Sahin, M.D., Ph.D.)
- Poster C3** Glucose transport dysfunction in Alzheimer's disease
David Henry Perlmutter, Medical Fellow, University of Rochester School of Medicine and Dentistry (Berislav V. Zlokovic, M.D., Ph.D.)
- Poster C4** Synaptic adhesion-like molecule 5 (SALM5) expression pattern in hippocampal neurons
Shila Azodi, Research Scholar, Texas Tech University Health Sciences Center School of Medicine (Robert J. Wenthold, Ph.D.)
- Poster C5** Loss of Parkin-induced mitophagy with disease-causing mutations
Derek Paul Narendra, Research Scholar, University of Michigan Medical School (Richard Youle, Ph.D.)
- Poster C6** Novel interaction of hereditary spastin paraplegia protein spartin (SPG20) with the endosomal sorting complex required for transport (ESCRT) protein hIST1
Rell Parker, Research Scholar, University of California, Davis, School of Veterinary Medicine (Craig Blackstone, M.D., Ph.D.)
- Poster C7** Functional evidence of neuroprotection through administration of an engineered anti-apoptotic fusion protein following acute spinal cord injury
Jayesh P. Thawani, Research Scholar, University of Michigan Medical School (Richard J. Youle, Ph.D.)
- Poster C8** Genome-wide association study for intracranial aneurysm
Nikhil R. Nayak, Medical Fellow, Yale School of Medicine (Murat Gunel, M.D.)
- Poster C9** Prestin upregulation increases outer hair cell electromotility and is associated with cell death
Christopher Cheng-Yu Liu, Medical Fellow, Baylor College of Medicine (John S. Oghalai, M.D., and William E. Brownell, Ph.D.)
- Poster C10** Molecular and functional changes associated with normal aging in the retina
Jessica Chang, Research Scholar, Duke University School of Medicine (Anand Swaroop, Ph.D.)
- Poster C11** Real-time analysis of EEG in the elucidation of volition
Logan Schneider, Research Scholar, University of Florida College of Medicine (Mark Hallett, M.D.)
- Poster C12** HermesC: low-power wireless neural recording system for freely moving primates
Paul Nuyujukian, Medical Fellow, Stanford University School of Medicine (Krishna Shenoy, Ph.D.)
- Poster C13** Behavioral and neural correlates of decision making after sleep deprivation: an fMRI study
Michelle Binder Jonelis, Medical Fellow, University of California, San Francisco, School of Medicine (Sean P.A. Drummond, Ph.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
ATRIUM

Poster Session D, 7:30–8:30 p.m.

page 91

Session Co-Chairs: Dean Wang and Maya Kasowski

- Poster D1** Amplifying the Wnt pathway to enhance bone regeneration
Steve Minear, Medical Fellow, Stanford University School of Medicine (Jill Helms, D.D.S., Ph.D., and Roel Nusse, Ph.D.)
- Poster D2** The mechanism and physiologic significance of osteoprotegerin repression by nuclear factor of activated T cells c1 during osteoclastogenesis
Rosalyn M. Sulyanto, Medical Fellow, Harvard School of Dental Medicine (Laurie H. Glimcher, M.D., and Antonios O. Aliprantis, M.D., Ph.D.)
- Poster D3** Characterization and expansion of human spermatogonial stem cells for the derivation of pluripotent cell lines
Raul I. Clavijo, Medical Fellow, University of California, San Francisco, School of Medicine (Renee A. Reijo Pera, Ph.D., and Paul J. Turek, M.D.)
- Poster D4** Conformational state of heat shock protein 90 governs binding of structurally diverse small-molecule inhibitors
Mari Johanna Tokita, Research Scholar, The Warren Alpert Medical School of Brown University (Len Neckers, Ph.D.)
- Poster D5** Role of the Pink1-Parkin in lipid-induced autophagy in skeletal muscle and liver cells
Sarah Elizabeth Rusk, Research Scholar, Case Western Reserve University School of Medicine (Michael N. Sack, M.D., Ph.D.)
- Poster D6** Parathyroid hormone-related protein overexpression protects chondrocytes subjected to injurious cyclic tensile strain
Dean Wang, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Rocky Tuan, Ph.D.)
- Poster D7** Metabolic stress of diabetes induces autophagy in the heart
Kari A. Wellnitz, Medical Fellow, University of Texas Health Science Center at Houston (Heinrich Taegtmeier, M.D., D. Phil.)
- Poster D8** Improved biocompatibility of implanted devices through seeding with adult stem cells
Michael Hodavance, Medical Fellow, Duke University School of Medicine (Bruce Klitzman, Ph.D., and W. Monty Reichert, Ph.D.)
- Poster D9** Analyzing variation in gene regulation in humans: global analysis of NF- κ B binding using ChIP-Seq in different individuals
Maya Kasowski, Medical Fellow, Yale School of Medicine (Michael Snyder, Ph.D.)
- Poster D10** Biomechanical control of microRNA expression and vascular homeostasis
Guadalupe Villarreal Jr., Medical Fellow, Harvard Medical School (Guillermo Garcia-Cardena, Ph.D.)
- Poster D11** Testing of a novel metabolite/transcript network for regulation of gluconeogenesis
Divakar Gupta, Medical Fellow, Duke University School of Medicine (Christopher B. Newgard, Ph.D.)

Session Co-Chairs: Regina LaRoque and Samantha Jordan

- 9:15 a.m.** A variant in long palate, lung, and nasal epithelium clone 1 is associated with cholera in a Bangladeshi population
Regina LaRoque, M.D., Early Career Awardee, Massachusetts General Hospital
- 9:30 a.m.** Predicting HIV-1 RNA level and loss of virologic suppression among HIV type-1-infected children receiving antiretroviral therapy in Tanzania
Susan D. Emmett, Medical Fellow, Duke University School of Medicine (Nathan M. Thielman, M.D.)
- 9:45 a.m.** A prospective study of tooth loss and cancer risk in a cohort of male smokers
Samantha Jordan, Research Scholar, Tufts University School of Dental Medicine (Christian C. Abnet, Ph.D.)
- 10:00 a.m.** Molecular characterization of ductal carcinoma in situ
Neil Desai, Medical Fellow, Yale School of Medicine (David F. Stern, Ph.D.)
- 10:15 a.m.** Patients with congenital heart disease and laterality defects exhibit ciliary dysfunction: a possible contributor to surgical outcomes?
Rachel A. Giese, Research Scholar, University of Texas Health Science Center at San Antonio (Cecilia W. Lo, Ph.D.)
- 10:30 a.m.** On-statin cholesteryl ester transfer protein mass and risk of recurrent coronary events: results from the PROVE IT-TIMI 22 study
Amit V. Khera, Medical Fellow, University of Pennsylvania School of Medicine (Daniel J. Rader, M.D.)

SCHEDULE OF PRESENTATIONS

WEDNESDAY
Room D-125

Neuroscience II

page 100

Session Co-Chairs: Joshua L. Roffman and Kimberley S. Mak

- 9:15 a.m.** *MTHFR* 677C→T disrupts prefrontal and dopaminergic function in schizophrenia
Joshua L. Roffman, M.D., Early Career Awardee, Harvard Medical School
- 9:30 a.m.** Why little Sally can't pay attention in class: functional neuroimaging of interictal attention deficits in childhood absence epilepsy
Matthew Vestal, Medical Fellow, Yale School of Medicine (Hal Blumenfeld, M.D., Ph.D.)
- 9:45 a.m.** Characterization of Neuregulin 3 (NRG3) and its role in schizophrenia
Wee-Tin Kao, Research Scholar, School of Medicine at Stony Brook University Medical Center (Daniel R. Weinberger, M.D.)
- 10:00 a.m.** Characterization of PCDH7 and GJA-1 in the formation of brain metastases
Kimberley S. Mak, Medical Fellow, Harvard Medical School (Joan Massagué, Ph.D.)
- 10:15 a.m.** Emboli extravasation is an alternative mechanism for cerebral microvascular recanalization
Carson K. Lam, Medical Fellow, Northwestern University, The Feinberg School of Medicine (Jaime Grutzendler, M.D.)
- 10:30 a.m.** Downstream mediators of SOX6 control over cortical interneuron development
Corinna Clio Zygourakis, Medical Fellow, Harvard Medical School (Jeffrey D. Macklis, M.D.)

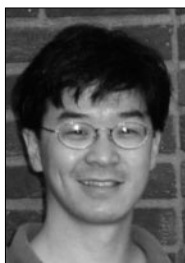
ABSTRACTS OF PRESENTATIONS

MONDAY
ROOM D-124

9:00 A.M.

Prognostic and predictive genetics of colorectal cancer via cancer genome sequence deconstruction

HANLEE P. JI, M.D., Early Career Awardee, Stanford University School of Medicine



H. P. Ji

■ Cancer development and clinically relevant tumor phenotype are the direct result of mutations in specific cancer genes in combination with other genomic aberrations such as copy number variations/aberrations and epigenetic changes affecting transcription. Many clinical studies have confirmed the clinical importance of mutations using a candidate gene approach and demonstrated associations between mutations and 1) overall aggressiveness of cancer as oftentimes seen in recurrence, 2) evidence of distal spread on initial presentation, and 3) response or resistance to specific therapies. Comprehensively identifying these mutations in cancer and drug target genes has enormous potential for genetic biomarker signatures that indicate prognosis and, in some cases, predict therapeutic response.

The advent of “next-generation” or “ultra-high-throughput” DNA sequencing technology is revolutionizing cancer genomics. These systems generate DNA sequences from a broad range of macromolecules and biological conditions up to the gigabase range (>1,000,000,000 nucleotides). To deconstruct cancer genomes at DNA sequence resolution for genetic biomarker discovery, my group is developing methods for targeted regional resequencing, genome-wide sequencing, and novel bioinformatic approaches, with a specific focus on resolving the clinical ambiguity of prognostic genetic markers in colorectal cancer.

Our strategy relies on highly scalable, sequence-specific methods of targeted DNA circularization. This approach allows resequencing of large numbers of candidate regions and simultaneously determining 1) somatic mutations, including deletions and insertions; 2) germline polymorphisms; and 3) copy number variations. By integrating these targeted methods with broader sequencing strategies such as transcriptome sequencing and paired-end interval analysis of cancer genomes, we can identify break points caused by intrachromosomal rearrangements such as genomic deletions, point mutations in exons and regulatory sequences and recombination sites. These mutations and genomic aberrations then are used to identify critical genes of interest that influence cancer clinical phenotype and are vetted as potential prognostic and predictive biomarkers.



C. P. GIACOMINI

9:15 A.M.

Creation of custom microarrays for identifying novel gene fusions in human malignancies

CRAIG P. GIACOMINI, Medical Fellow, Stanford University School of Medicine

Mentor: Jonathan R. Pollack, M.D., Ph.D., Stanford University School of Medicine

■ Chromosomal rearrangements creating pathogenic gene fusions are common and indeed often pathognomonic in hematologic and mesenchymal tumors. These fusion genes are used clinically for diagnosis and prognostication, and can be important targets for therapy, for example, imatinib (Gleevec) targeting *BCR/ABL*, and all-*trans* retinoic acid (ATRA) targeting *PML/RARA*. In contrast, fusion genes have been considered rare in common epithelial malignancies like breast, lung, and colon carcinomas. We hypothesize that gene fusions are present but remain unidentified in these malignancies because cytogenetic analysis of epithelial tumors is challenging and because rampant chromosome instability masks key recurrent rearrangements. New methods are, therefore, needed to identify novel gene fusions in these cancer subtypes.

The goal of this study was to explore novel microarray-based techniques for gene fusion detection. Toward this goal, we designed a custom tiling microarray as well as a fusion junction microarray for the detection of these oncogenic chromosomal rearrangements. For validation, we demonstrated that these platforms could detect known gene fusions, including *BCR/ABL*, *NPM/ALK*, and *TMPRSS2/ERG*, in a series of positive control cancer cell lines and clinical specimens. We then used the custom tiling array to screen a series of 64 cancer samples ranging among various subtypes to identify novel gene fusions. Among our findings, we identified a novel breakpoint in the *TMPRSS2/ETV4* gene fusion in a prostate cancer clinical specimen. We also found various gene fusions known to exist in particular cancer subtypes but not previously identified in specific cell lines. For example, we identified a potential *NOTCH1* gene fusion and *PDGFRA* gene fusion previously unidentified in two leukemia cell lines, defining new cell line models for studying these oncoproteins. Screening additional samples should identify novel pathogenic gene fusions, improving our understanding of the molecular basis of human cancers.

9:30 A.M.

Changes in diffusion of macromolecules in human blood clots resulting from exposure to high-intensity focused ultrasound

GUY C. JONES, Research Scholar, University of Medicine and Dentistry of New Jersey–New Jersey Medical School

Preceptor: Bradford J. Wood, M.D., Clinical Center, National Institutes of Health

■ Deep vein thrombosis (DVT) accounts for, or results from, hundreds of thousands of hospital visits every year. DVT causes significant morbidity and mortality as it can progress to pulmonary embolism (PE) and occasionally stroke. Anti-coagulation therapy, especially heparin, warfarin, and enoxaparin, is the most common treatment for DVT. This treatment is effective at slowing the advancement of the disease but often fails to eliminate the source of the problem, which is necessary to prevent permanent damage to venous valves and the vascular bed.

Ultrasound, by itself and adjunctly with thrombolytic drugs, has been studied as a means to treat DVT. Studies have demonstrated that pulsed high-intensity focused ultrasound (pulsed-HIFU) can significantly enhance tPA-mediated thrombolysis both in vivo and in vitro. It has been hypothesized that pulsed-HIFU may cause structural changes in clots leading to increased penetration of tPA, thereby improving tPA's thrombolytic potential. Our goal is to investigate whether pulsed-HIFU treatment of human blood clots will allow improved diffusion of macromolecules through human blood clots in vitro.

Flattened human blood clots will be created from the venous blood of healthy volunteers mixed with dextrans of different molecular weights. These clots will be allowed to form in penrose tubing and then will be treated with pulsed-HIFU at exposures shown to enhance thrombolytic effects when combined with tPA. Following HIFU exposures, clots will be removed from the tubing and placed on slides. Fluorescence recovery after photobleaching (FRAP) analysis will be run on each sample using confocal microscopy to obtain diffusion coefficients for each dextran size. FRAP will be run on the same dextran sizes in water, agarose, and clots not exposed to HIFU to provide a means of comparison.

9:45 A.M.

Can dynamic contrast-enhanced multidetector computed tomography accurately measure fluid flow velocity?

LISA L. CHU, Medical Fellow, University of California, San Francisco, School of Medicine

Mentor: Benjamin M. Yeh, M.D., University of California, San Francisco

■ Multidetector computed tomography (MDCT) angiography is well-tolerated and provides high temporal and spatial resolution for the evaluation of arterial disease. However, it focuses almost exclusively on anatomy and provides little if any information on physiology, such as flow velocity and turbulence. Clearly, form is related to function, and understanding flow velocity within a vascular segment could potentially enhance the value of MDCT angiography for patient care by further characterizing the nature and severity of arterial disease. Our research tests a novel dynamic contrast-enhanced MDCT method for measuring fluid flow velocity.

We constructed a CT phantom of half-inch-diameter tubing with water flow serially set at seven increasing velocities (from 8.6 to 54.2 cm/s). For each velocity, after contrast injection, cine-MDCT images of the tubing were obtained to record the transit of the contrast bolus by using three protocols: 1) 16- × 2.5-mm-thick slices every 0.25 s, 2) 8- × 5.0-mm-thick slices every 1.0 s, and 3) 8- × 5.0-mm-thick slices every 2.0 s. Flow velocity was calculated in two ways: 1) trendline slope of the time to peak (TTP) enhancement of each slice plotted against its distance from the first slice, and 2) trendline slope of the mean time (MT, mean of time weighted by attenuation) of each slice plotted against its distance from the first slice. We found that flow velocities as determined by MDCT using a single contrast bolus have excellent correlations ($r > 0.90$) and agreement to reference standards.

Subsequent experiments in phantoms with varied tubing diameters and with pulsatile flow gave similarly promising but more variable results. Studies to test this method in vivo are currently ongoing. Our study is the first to measure flow velocity using MDCT imaging of a single contrast bolus and provides a framework for future dynamic imaging of blood flow at CT angiography.



G. C. JONES



L. L. CHU

MONDAY
ROOM D-124

10:00 A.M.

Insulin-sensitive fusion of vesicles containing glucose transporter with the plasma membrane can be detected and characterized by total internal reflection fluorescence microscopy

ALLAN S. MABARDY, Medical Fellow, University of Massachusetts Medical School

Mentor: Michael Czech, Ph.D., University of Massachusetts Medical School

■ Whole-body glucose homeostasis requires insulin-stimulated glucose uptake in muscle and adipose tissue, mediated by the translocation of the glucose transporter GLUT4 from intracellular endosomes to the plasma membrane (PM). Though muscle is responsible for the bulk of glucose disposal, knockout of GLUT4 in adipocytes results in a diabetic phenotype in mice. Recent studies suggest that fusion of GLUT4 vesicles with the PM may be a critical insulin-regulated step, but underlying mechanisms are unknown. To study the kinetics of fusion events in 3T3-L1 adipocytes, we expressed a surrogate GLUT4 protein, labeled cytosolically with a red fluorescent protein (RFP) variant and lumenally with a pH-sensitive green fluorescent protein, ecliptic pHluorin.

Fusion events can be analyzed using total internal reflection fluorescence (TIRF) microscopy, which illuminates the cell surface to a depth of 100 nm. Fusion candidates were identified by a sudden increase in pHluorin emission due to exposure to the less acidic extracellular environment. Since vesicles are smaller than the pixels of our camera, quantifying pixel intensity along a line drawn through any fusion event reveals a distinct Gaussian profile, and as the fluorophore is distributed laterally on the PM, this curve flattens, thus differentiating a fusing vesicle from a vesicle retreating into the cytosol. Our analyses reveal a stimulatory effect of insulin on the rate of fusion events, thus validating the methodology.

We are in the process of devising an algorithm to quantify the kinetic parameters of presumed tethering, docking, and fusion in the presence and absence of insulin, as well as events where a fusion pore forms but full fusion does not occur. These analyses will determine insulin-regulated steps in vesicle dynamics and will allow us to further investigate the role of proposed mediators of GLUT4 vesicle fusion.



A. S. MABARDY



A. D. BANSAL

10:15 A.M.

Expansion of the rat inner medullary collecting duct phosphoproteome and quantitative mass spectrometry of urea transporter phosphorylation in response to vasopressin

AMAR D. BANSAL, Research Scholar, New York University School of Medicine

Preceptor: Mark A. Knepper, M.D., Ph.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ Phosphorylation is a major posttranslational modification that can alter cellular processes related to protein structure, function, trafficking, or signaling. Tandem mass spectrometry (LC-MS/MS) enables large-scale identification of phosphoproteomic data, which can subsequently be used to investigate the role of individual proteins.

To augment the traditional LC-MS/MS approach for shotgun proteomics, we utilized a combination of strong cation exchange (SCX) chromatography, as well as multiple search algorithms (SEQUEST and InsPecT), to expand the size of the rat inner medullary collecting duct (IMCD) phosphoproteome from 401 to 693 proteins (filtered to a 2% false discovery rate based on target-decoy analysis).

Included in this new dataset were three novel phosphorylation sites (Ser-10, Ser-62/63, and Ser-84) and one previously identified phosphorylation site (Ser-486) on the urea transporter UT-A1. Next, we tested whether phosphorylation on these sites was dependent on treatment with the vasopressin analog dDAVP.

Treatment with dDAVP had no significant effect ($P > 0.05$) on the abundance of phosphorylation on Ser-10 and Ser-62/63. However, dDAVP treatment caused a 5.9-fold increase in abundance of Ser(P)-84 ($P < 10^{-6}$) and a 5.7-fold increase in abundance of Ser(P)-486 ($P < 0.01$). Stable isotopically labeled peptides were also used to verify the retention time of MS¹ precursor ions used for quantification of relative peptide abundances.

Additional studies, such as immunocytochemistry using custom-designed phosphospecific antibodies, will be performed to elucidate relationships between phosphorylation on these sites and UT-A1 trafficking to the apical plasma membrane.

10:45 A.M.

Sensing danger within: role of an endogenous alarm molecule in mediating inflammation of the liver

ALLAN TSUNG, M.D., Early Career Awardee, University of Pittsburgh School of Medicine

■ Ischemic tissues require mechanisms to notify the immune system of the impending cell damage and possible breach of tissue integrity. Cells of the innate immune system are activated following ischemic injury to initiate tissue repair processes and to provide defense against microbial invasion. Excessive activation can lead to exaggerated local and systemic inflammation, which may extend the tissue damage. We propose that a key link between the initial damage to cells and the activation of inflammatory signaling involves endogenous danger signals from ischemic cells. The purpose of our studies was to determine how a nuclear protein, high mobility group box-1 (HMGB1), is mobilized and can act as an alarm molecule in response to liver ischemic stress.

We found that HMGB1 is upregulated in cultured hepatocytes by hypoxia and hepatic ischemia and reperfusion (I/R) injury in vivo. Inhibition of HMGB1 activity with neutralizing antibody significantly decreased liver damage and suppressed the activation of inflammatory cascades after I/R, whereas administration of recombinant HMGB1 worsened I/R injury. To determine the receptor involved in HMGB1-mediated inflammation, we examined the role of Toll-like receptor 4 (TLR4). TLR4 knockout mice exhibited less damage in the hepatic I/R model compared to their wild-type counterparts. In addition, HMGB1 blockade failed to provide protection in TLR4 knockout mice, but successfully reduced damage in wild-type mice.

Taken together, these results demonstrate that a nuclear protein, HMGB1, is an early mediator of inflammation and organ damage after ischemic liver injury and implicates TLR4 as one of the receptors involved in the process. Interaction of HMGB1 with TLR4 could provide a critical link between tissue damage and activation of the innate immune response, and understanding these mechanisms may lead to potential therapeutic strategies to minimize organ damage in a variety of inflammatory clinical settings.

11:00 A.M.

Zymosan-mediated inflammation impairs in vivo reverse cholesterol transport in mice

PRIYA MALIK, Medical Fellow, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentor: Jonathan D. Smith, Ph.D., Lerner Research Institute, Cleveland Clinic Foundation

■ Atherosclerotic coronary artery disease (CAD) remains a leading cause of death despite the advent of new lipid-lowering medications. High-density lipoprotein (HDL) is known to prevent development of CAD by facilitating transport of cholesterol from the periphery to the liver for excretion in bile, a process known as reverse cholesterol transport (RCT). However, oxidative damage was recently shown to cause HDL dysfunction by impairing its major protein apolipoprotein A1 (ApoA1). Inflammation and the leukocyte enzyme myeloperoxidase (MPO) may be sources of this oxidative damage. To determine the link among inflammation, oxidative damage, and HDL dysfunction in vivo, we investigated the effects of zymosan, a yeast glucan that induces inflammation and oxidative burst, on in vivo reverse cholesterol transport.

We measured RCT by injecting bone marrow macrophages loaded with ^3H -AcLDL (to resemble foam cells) subcutaneously and assessed radioactivity in the plasma and feces. Inflammation, induced by a 2-mg intraperitoneal injection of zymosan, caused a 22% ($\pm 3\%$) decline of plasma RCT and 21% ($\pm 4\%$) decline of cumulative fecal RCT over a 3-day period. Zymosan also caused a 20-fold increase in serum amyloid A levels, but no change in ApoA1 levels. Plasma from mice with inflammation further had a 22% ($\pm 5\%$) decreased ex vivo ability to accept cholesterol from cultured macrophages laden with ^3H -cholesterol. However, zymosan-mediated inflammation still impaired RCT in MPO knockout mice, suggesting an MPO-independent mechanism of RCT impairment in our model.

Our model of inflammation induced via the yeast glucan zymosan impairs in vivo RCT to plasma and feces by reducing the ability of plasma to accept cholesterol. Further studies are being performed to determine the mechanism of this impairment by examining whether oxidative modification of ApoA1 is the culprit behind this decreased ability of plasma to accept cholesterol.

MONDAY
ROOM D-124



A. TSUNG



P. MALIK

MONDAY
Room D-124

11:15 A.M.

Molecular mechanism of β -amyloid inhibition of nitric oxide signaling

HUBERT SHIH, Research Scholar, David Geffen School of Medicine at UCLA

Preceptor: David D. Roberts, Ph.D., National Cancer Institute, National Institutes of Health

■ Nitric oxide (NO) is a bioactive gas produced constitutively by many cell types and is involved in various functions. Among its most well known roles is as a regulator of vessel tone in the cardiovascular system. It has been previously shown that β -amyloid ($A\beta$), a peptide extensively studied in Alzheimer's disease, is a component in human atherosclerotic plaques. As $A\beta$ has been demonstrated to inhibit NO signaling and NO has been shown to have a protective role via its regulation of vascular tone, we seek to understand the molecular mechanism of how $A\beta$ affects NO signaling inhibition to contribute to the understanding of atherosclerosis pathophysiology.

Thrombospondin-1 (TSP1) is a well-studied matrix glycoprotein that shares a number of similarities with $A\beta$. TSP1 is known to inhibit NO signaling by interacting with a cell surface receptor complex consisting of the integral membrane proteins CD36 and CD47. TSP1 can provide a basis for understanding the effects of $A\beta$ on NO signaling. We hypothesize that $A\beta$ inhibition of NO signaling requires CD47 and CD36.

To investigate this, we will look at the effect that pretreatment with $A\beta$ has when various cell lines, including human and porcine vascular smooth muscle cells, are treated with NO. Endpoints that will be examined as a measure of NO signaling include measuring cyclic guanosine monophosphate (cGMP) levels and vasodilator-stimulated phosphoprotein (VASP) phosphorylation, as well as examining changes in cell morphology as determined by visual examination and electrical impedance. The specific cellular mechanism by which $A\beta$ inhibits NO signaling will be pursued using agents such as activators of peroxisome proliferator-activated receptor- γ (PPAR γ) and CD36 morpholinos, which influence the expression of CD36, and also using CD47 null cells. Preliminary data suggest that both CD47 and CD36 are involved in $A\beta$ inhibition of NO signaling.



H. SHIH



L. BRAUNSTEIN

11:30 A.M.

Immune surveillance is mediated by the antiangiogenic activity of thrombospondin-1

LIOR BRAUNSTEIN, Medical Fellow, Harvard Medical School

Mentor: Sandra W. Ryeom, Ph.D., Harvard Medical School, Children's Hospital Boston

■ Immune surveillance is thought to play a key role in the early suppression of tumor growth via mechanisms that include the cytotoxic function of lymphocytes. Data suggest, however, that other mechanisms may also contribute. Antiangiogenic surveillance is the process by which endogenous inhibitors of angiogenesis are used to counteract the increase in proangiogenic factors during the early stages of tumor progression. Numerous endogenous angiogenesis inhibitors have been discovered; of these, thrombospondin-1 (TSP-1) is among the most potent.

Our studies suggest that TSP-1 is expressed most highly in the spleen among all organs and that this expression is largely confined to the CD4+ compartment. Consistent with these findings, TSP-1 expression is almost undetectable in the spleens of immunocompromised SCID mice that lack T and B cells. Adoptive transfer and bone marrow transplant studies with *wild-type* and *Tsp-1^{-/-}* splenocytes and CD4+ T cells demonstrated that suppression of tumor growth was significantly abrogated in the absence of TSP-1. The upregulation of TSP-1 after CD4+ T cell activation appears critical for the attenuation of the earliest stages of tumor angiogenesis. Furthermore, tumor suppression as a consequence of immunotherapy with CD4+ T cells is ineffective in the absence of TSP-1.

Taken together, our data suggest that TSP-1 plays an important role in T-cell-mediated immune surveillance. By elucidating how immunological mechanisms inhibit tumor angiogenesis, a novel therapeutic modality may be developed whereby the tumor-targeting potential of this immune population is exploited for its antiangiogenic and anti-tumor potential.

11:45 A.M.

LFA-1-mediated, HuR-dependent stabilization of VEGF mRNA in macrophages

YASHA MODI Medical Fellow, Yale School of Medicine

Mentor: Jeffrey R. Bender, M.D., Yale School of Medicine

■ Activated macrophages, via the secretion of pro-angiogenic factors, may modulate the angiogenic response to inflammation. Several of these factors, including vascular endothelial growth factor (VEGF), are encoded by inherently unstable mRNA transcripts containing AU-rich elements (AREs) in their 3' untranslated region. During stress events, the half-lives of these mRNAs must be prolonged to allow for significant protein production and an adequate angiogenic response. This laboratory has demonstrated that engagement of the β 2-integrin receptor, LFA-1, leads to stabilization of ARE-bearing mRNAs in T lymphocytes through a nuclear-to-cytoplasmic translocation of the RNA-binding protein HuR. Here, we address whether β 2-integrin engagement stabilizes VEGF mRNA in macrophages in vitro and at sites of angiogenesis via a HuR-dependent mechanism.

To determine whether β 2-integrin engagement regulates VEGF mRNA stability, we adhered primary mouse bone marrow-derived macrophages to ICAM-1 (β 2-integrin ligand) for 3 hours. VEGF mRNA degradation was measured by quantitative RT-PCR after transcriptional inhibition. In the cells bound to ICAM-1, the VEGF mRNA remained stable, whereas in control cells, more than half of the VEGF mRNA degraded within 60 minutes.

To study inflammatory angiogenesis in vivo, we used a model of subcutaneous implantation of polyvinyl alcohol (PVA) sponges to assess differences in wild-type and "macrophage-specific" HuR knockout (HuR^{fllox/fllox}LysM-Cre) mice. Flow cytometry analyses of cells extracted from PVA sponges at 1–4 weeks showed essentially equal recruitment of F4/80⁺ cells in wild-type and HuR knockout mice. Immunofluorescence staining of excised PVA sponges confirmed equal macrophage recruitment and localization but revealed a reduction of VEGF production. Preliminary results indicate that formation of CD31⁺ microvessels in HuR knockout mice is also decreased.

We conclude that macrophage β 2-integrin engagement results in stabilization of VEGF and that expression of HuR in macrophages is required for neovascular responses. These findings are relevant to understanding the posttranscriptional regulatory mechanisms of inflammatory angiogenesis.

Noon

Vascular endothelial growth factor has distinct roles during heart development

GENE KEW MA, Medical Fellow, Stanford University School of Medicine

Mentor: Ching-Pin Chang, M.D., Ph.D., Stanford University School of Medicine

■ Tissues of the developing heart respond to a coordinated series of extracellular signals to form the many distinct anatomical features of the mature organ. Disruption of these signaling networks contributes to congenital heart disease, the most common class of birth defects. Among these signals, vascular endothelial growth factor (VEGF) signaling is critically important; however, its specific temporal roles remain poorly defined.

Using chemical-induced expression of inhibitors of VEGF in transgenic mouse embryos, we have characterized time windows during which VEGF has three distinct functions during cardiac morphogenesis. (1) Early during development, around embryonic day 9.5 (E9.5), a subset of endocardial cells undergoes an epithelial-to-mesenchymal transformation (EMT) to populate structures called endocardial cushions, which subsequently form the heart valves. Induced expression of a soluble inhibitor of VEGF signaling (sFlt) at E9.5 did not prevent initial delamination of endocardial cells undergoing EMT. However, they failed to differentiate fully into mesenchymal cells, retaining expression of endocardial markers such as PECAM-1 and NFATc1. (2) Later during development, beginning at E11.5, the endocardial cushions undergo a dynamic remodeling and elongation process to form long, delicate valve leaflets. Blocking VEGF signaling by expression of a second inhibitor of VEGF (VEGFR2T) during this period prevented valve elongation. (3) Expression of VEGFR2T at E11.5 also caused cellular apoptosis specifically within the interventricular septum and prevented endothelialization of the septum.

Our findings reveal varied roles for VEGF at different stages and in different tissues of the developing heart. VEGF regulates cellular differentiation during the formative stages of valve development and later morphogenetic changes required for valve maturation. Additionally, VEGF coordinates appropriate cell survival to support ventricular chamber septation. These results provide a framework to understand how perturbations of VEGF signaling contribute to congenital heart defects.



Y. MODI



G. K. MA

MONDAY
Room D-125

9:00 A.M.

RBP-R2: a potential RBP4 receptor and retinol transporter in liver, adipose tissue, and gut

TIMOTHY E. GRAHAM, M.D., Early Career Awardee, Beth Israel Deaconess Medical Center



T. E. GRAHAM

■ Insulin resistance plays an important role in the pathogenesis of type 2 diabetes and cardiovascular disease. Obesity is the most frequent cause of insulin resistance. We previously reported increased production of serum retinol binding protein (RBP4) in visceral adipose tissue and increased RBP4 levels in serum of insulin-resistant humans. In animal models, we found that elevated serum RBP4 acts systemically to cause insulin resistance in muscle and increased glucose production in liver. RBP4 is the sole specific transport protein for retinol (Vitamin A) in blood, but it is not known whether altered delivery or metabolism of retinol mediates insulin resistance caused by RBP4. A recently identified high-affinity RBP4 receptor and transmembrane retinol transporter, Stra6/RBP-R1, is expressed primarily in retina and brain. We discovered a second potential RBP4 receptor that we have named RBP-R2. We hypothesize that RBP-R2 mediates RBP4 binding and/or retinol uptake in target tissues of insulin action. Quantitative RT-PCR was used to determine RBP-R2 expression in different tissues in mice. The ability of RBP-R2 to mediate retinol uptake from RBP4 was tested in cell culture. We found that RBP-R2 is most highly expressed in liver, intestine, and adipose tissue of normal mice, and expression of RBP-R2 is dramatically increased in adipose tissue of mice with obesity and insulin resistance. Overexpression of RBP-R2 in rat H4IIE hepatocytes confers increased retinol uptake from holo-RBP4. These findings suggest that RBP-R2 may mediate RBP4-dependent retinol uptake in tissues involved in retinol absorption (e.g., intestine) or storage (e.g., liver and adipocytes); the findings further support a role for RBP-R2 in obesity-related changes in adipose tissue. Our laboratory is developing mice with *Cre/loxP*-mediated genetic deletion of RBP-R2 in different tissues to determine how tissue-specific expression of RBP-R2 may contribute to whole-body retinol homeostasis and insulin-glucose homeostasis in vivo.



G. NARLA

9:15 A.M.

An oncogenic splice variant of the Kruppel-like factor 6 (KLF6) tumor-suppressor gene promotes prostate cancer progression and metastasis

GOUTHAM NARLA, M.D., Ph.D., Early Career Awardee, Mount Sinai School of Medicine

■ Metastatic prostate cancer (PCa) is a leading cause of cancer death in men. It is estimated that 234,460 men will be diagnosed with the disease and 27,350 will die this year alone. Although gene loci, candidate genes, and risk factors for subsets of prostate cancer have been identified, the molecular mechanisms underlying the transition from localized to metastatic PCa have yet to be fully elucidated. The identification of individual genes and biomarkers predictive of the clinical behavior of any given prostate tumor remains an urgent priority. Given the clinical heterogeneity in the disease, appropriate molecular classification of the disease to improve patient stratification for treatment and to develop novel targeted molecular therapies is of utmost importance. Accumulating evidence suggests that the tumor-suppressor gene Kruppel-like factor 6 (KLF6) and its oncogenic splice variant (KLF6-SV1) play an important role in the development and progression of cancer. Expression array studies have identified changes in KLF6 gene expression as predictive of clinical outcome in prostate cancer. The present studies were directed toward exploring the role of KLF6-SV1 in prostate cancer development and progression. Quantitative real-time PCR analysis and immunohistochemical studies of tumor samples demonstrated that KLF6-SV1 expression was specifically upregulated in hormone-refractory metastatic PCa. Functional studies using both cell culture and three complementary mouse model systems demonstrated that KLF6-SV1 overexpression resulted in increased cellular invasion, prostate cancer progression, and increased metastasis to lymph nodes, bone, and brain. Targeted reduction of KLF6-SV1 using RNA interference (RNAi) resulted in the induction of apoptosis in cultured prostate cancer cell lines and suppressed tumor growth in mice. Combined, these studies demonstrate that KLF6-SV1 expression levels in PCa tumors can predict the metastatic potential of localized PCa and that KLF6-SV1 may represent a novel therapeutic target in the treatment of metastatic prostate cancer.

9:30 A.M.

HIF-2 α in acute and malignancy-associated inflammation**EMILY P. WILLIAMS**, Medical Fellow, University of Pennsylvania School of Medicine

Mentor: M. Celeste Simon, Ph.D., Howard Hughes Medical Institute, University of Pennsylvania School of Medicine

■ Cellular adaptation to hypoxia is critical in normal development and tissue maintenance, but also plays an important role in the pathophysiology of many disease states, including wounds and solid tumors. Cells exposed to reduced oxygen tension stabilize the hypoxia-inducible factors, HIF-1 and HIF-2, which regulate transcription of genes required for response to hypoxic stress. HIF-1 α is required for normal macrophage function, but less is known about the role of HIF-2 α in the monocyte lineage. HIF-2 α is highly expressed in human tumor-associated macrophages (TAMs); additionally, the presence of TAMs has been correlated with poor clinical outcome in a variety of human cancers. We were interested in studying the role of HIF-2 α in macrophages in acute inflammation and in the setting of inflammation associated with solid tumors.

Our lab generated a mouse model with HIF-2 α deleted specifically in the myeloid lineage. Macrophages generated from the bone marrow of these myeloid HIF-2 α null mice show significantly reduced secretion of the inflammatory cytokines IL-1 β , IL-12, and IL-6 and the chemokine CXCL2 when activated and exposed to hypoxia. These macrophages also have significant defects in migration and invasion in vitro. Mice with HIF-2 α null myeloid cells show resistance to LPS-induced sepsis and reduced ability to mount inflammatory responses to subcutaneous and intraperitoneal irritants. When induced to develop hepatocellular carcinoma, the myeloid HIF-2 α null mice have significantly fewer TAMs in their hepatic tumors. A reduced number of TAMs correlates with a lower average mitotic index and tumor grade in hepatic tumors of the mutant mice compared to those of control mice.

These data demonstrate the requirement of HIF-2 α for normal macrophage cytokine secretion, migration, and invasion, which contribute to acute inflammatory reactions and the immune response to solid tumors. Current studies are focused on better understanding the mechanisms through which HIF-2 α contributes to macrophage function.

9:45 A.M.

Leflunomide activates the Notch pathway, leads to carcinoid cancer cell cycle arrest, and represents a novel potential therapeutic option**MACKENZIE R. COOK**, Medical Fellow, Duke University School of Medicine

Mentor: Herbert Chen, M.D., University of Wisconsin–Madison

■ Carcinoid cancers are highly metastatic and often associated with debilitating endocrinopathies. Activation of the Notch signaling pathway has been shown to suppress carcinoid growth and alter the malignant phenotype. A recent quantitative high-throughput screen (qHTS) designed to identify novel Notch activators identified leflunomide (LFN), currently in clinical use for rheumatoid arthritis. As there are limited therapeutic options for metastatic disease, this study was designed to investigate the effects of LFN, and its major metabolite teriflunomide (TEF), on the growth and malignant phenotype of carcinoid cancer cells.

Human gastrointestinal (BON) and pulmonary (H727) carcinoid cells treated with either LFN or TEF were analyzed by Western blot for Achaete-Scute complex-like 1 (ASCL1), chromogranin-A (CgA), p21, p15, and cyclin B1. A MTT colorimetric growth assay quantified the number of viable cells after treatment for 0–6 days. Flow cytometric analysis with propidium iodide staining was utilized to investigate cell cycle progression. Transient transfection with a CBF1-luciferase construct was used to assay functional Notch pathway activation.

Treatment with LFN and TEF led to an increase in luciferase activity, a measure of Notch pathway activation. This was associated, in both cases, with a dose-dependent reduction in ASCL1, a downstream effector of the Notch pathway. Significant growth suppression was observed by MTT. Western blot for markers of cell cycle progression as well as flow cytometry indicate cell cycle arrest at the G1/S checkpoint.

These data validate our qHTS and demonstrate that LFN and TEF are novel Notch pathway activating compounds, capable of suppressing growth and altering the malignant carcinoid phenotype. As these effects are observed at doses that are achievable and minimally toxic in humans, LFN and TEF represent novel therapeutic options and warrant further study in vivo.



E. P. WILLIAMS



M. R. COOK

MONDAY
ROOM D-125

10:00 A.M.

High-throughput screening identification of an inducer of 15-hydroxyprostaglandin dehydrogenase, a suppressor of human colon cancer

LUCY LE HE, Medical Fellow, Case Western Reserve University School of Medicine

Mentor: Sanford D. Markowitz, M.D., Ph.D., Howard Hughes Medical Institute, Case Western Reserve University School of Medicine

■ 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) is an enzyme that our laboratory has hypothesized to be a suppressor of colon tumorigenesis. This enzyme catalyzes the endogenous breakdown of prostaglandins and is highly expressed in normal colon epithelium but lost in colon cancer. Our laboratory demonstrated that 15-PGDH has marked *in vivo* activity as a colon cancer tumor suppressor. We concluded that downregulation of 15-PGDH is a key step in colon tumorigenesis and agents that reactivate 15-PGDH expression could provide therapeutic or preventive benefits against colon cancer. We constructed colon cancer reporter cell lines where the Renilla luciferase (hRL) fluorescent reporter is introduced into the 15-PGDH locus and whose expression is driven by the 15-PGDH promoter. Induction of 15-PGDH can be easily detected via commercial Renilla luciferase assays and confirmed via Western blotting. To cover the spectrum of colon cancers, we used a microsatellite stable (MSS) cell line, Vaco9M, and a microsatellite unstable (MSI) cell line, LS174T, for these 15-PGDH-hRL fusion constructs.

Our laboratory hypothesized that the Wnt signaling pathway may play a role in PGDH downregulation. Using short interfering RNA (siRNA) to β -catenin reinduced PGDH expression in the LS174T-hRL constructs as seen by Renilla luciferase assay and confirmed by Western blotting. Treatment of the Vaco9M-hRL constructs with an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, erlotinib (Tarceva), has also been able to reinduce 15-PGDH expression. These results implicate different biological signaling pathways as being involved in PGDH regulation, in different models of colon cancer. Searching for drug targets in either pathway via traditional methods is an arduous task. However, with our 15-PGDH-hRL constructs, we can readily utilize high-throughput screening of various siRNA libraries, small-molecule libraries, and the tyrosine kinome. Candidate agents that reconstitute 15-PGDH expression in our constructs may be novel chemotherapeutic or chemopreventive anti-colon-cancer drugs.



L. L. HE



L. M. GUENTHER

10:15 A.M.

Targeting the MET tyrosine kinase receptor to inhibit osteosarcoma metastasis

LILLIAN M. GUENTHER, Research Scholar, State University of New York Downstate Medical Center College of Medicine

Preceptor: Chand Khanna, D.V.M., Ph.D., National Cancer Institute, National Institutes of Health

■ MET, a tyrosine kinase receptor for hepatocyte growth factor (HGF), is mutated and activated in many human cancers, suggesting that its dysregulation is involved in tumorigenesis and metastasis. Osteosarcoma is the most common malignant pediatric bone tumor in the United States. MET is overexpressed in most human osteosarcomas. We hypothesized that MET is constitutively activated in osteosarcoma and that this activation is central to metastatic potential. Utilizing PHA665752, a small-molecule inhibitor of MET, we investigated whether MET inhibition reduces features of the malignant phenotype in a panel of osteosarcoma cell lines, thereby establishing it as a potential therapeutic target.

Quantitative electrochemiluminescence (ECL) demonstrated variable levels of total MET expression across a panel of human osteosarcoma cell lines and tumor samples. Phospho-MET expression was present in two of six patient-derived cell lines, correlated with high levels of total MET, and was downregulated with PHA665752 treatment. When treated, MNNG-HOS, a carcinogenically transformed MET-activated metastatic line, showed a 44% decrease in migration ($\pm 3\%$), an 81% decrease in invasion ($\pm 1\%$), and decreased cell motility over 72 hours. Additionally, MNNG-HOS cells treated with PHA665752 exhibited decreased ability to form clusters in *ex vivo* lung culture. Patient-derived cell lines demonstrating phospho-MET expression by ECL showed a 38% decrease in migration ($\pm 8\%$) and decreased motility when treated with PHA665752 compared to untreated controls.

Results demonstrate activation of MET in a subset of osteosarcoma patient-derived cell lines. *In vitro* assays demonstrate sensitivity of the MET-activated cell line, MNNG-HOS, as well as two of six patient-derived lines, to PHA665752. Genomic sequencing is underway to identify activating mutations in MET, as well as work to evaluate HGF ligand expression. Based on these studies, inhibition of MET could be a potentially effective therapeutic intervention for the treatment of malignant osteosarcoma and should be further investigated.

10:45 A.M.

Role of apelin-APJ signaling in the vasculature

HYUNG J. CHUN, M.D., Early Career Awardee, Stanford University School of Medicine

■ Cardiovascular diseases account for over 7 million deaths worldwide each year. Study of the signaling mechanisms that are involved in the progression of the associated vascular pathology will shed insights into potential novel therapeutic targets. APJ is a G protein-coupled receptor (GPCR) with a significant homology to the angiotensin II type 1 receptor. Its ligand apelin is a potent inotrope with vasodilatory properties that are in part mediated by nitric oxide. We hypothesize that the apelin-APJ signaling pathway plays a critical role in maintaining homeostasis of both the systemic and pulmonary vasculature, and that modulation of apelin signaling may serve a therapeutic role in cardiovascular disease.

Our recent findings have demonstrated inhibition of angiotensin II signaling by the apelin-APJ pathway, leading to dramatic inhibition of disease processes mediated by angiotensin II, including atherosclerosis and aortic aneurysms. This is in part mediated by direct antagonism of angiotensin II signaling by the apelin-APJ pathway. In the pulmonary vasculature, we have found that mice with perturbation of the apelin-APJ pathway develop worsening pulmonary hypertension. We have also found that apelin expression is markedly decreased in humans with and animal models of pulmonary hypertension.

We have demonstrated that the apelin-APJ pathway can ameliorate disease processes involving both the systemic and the pulmonary vasculature. Better understanding of the apelin-APJ pathway will provide further insights into the potential therapeutic benefits of this pathway in vascular wall disease.

11:00 A.M.

Pullulan-deferoxamine delivery film for targeted ischemic preconditioning

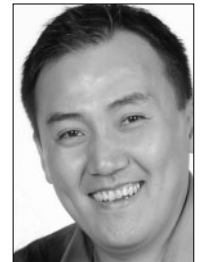
MICHAEL G. GALVEZ, Medical Fellow, Stanford University School of Medicine

Mentors: Geoffrey C. Gurtner, M.D., and Amato J. Giaccia, Ph.D., Stanford University School of Medicine

■ Ischemic preconditioning (IPC) is a phenomenon whereby a brief episode of ischemia-reperfusion (I/R) triggers potent mechanisms to protect tissue from subsequent sustained I/R. The intentional creation of IPC before flap transposition results in increased ischemic tolerance and improved flap survival. However, flap necrosis continues to be a challenging problem, especially in the setting of diabetes and aging, because of inadequate neovascularization and I/R-induced cell death. Deferoxamine (DFO) increases hypoxia inducible factor-1 (HIF-1), a potent transcription factor that can be effective for pharmacological IPC. Covalently conjugating DFO to pullulan, a biodegradable carbohydrate, was investigated for enhanced IPC in flap tissue before transposition. Specifically, delivery of DFO from pullulan films was compared to DFO saline injections. A peninsular skin flap was elevated dorsally on C57Bl/6 mice. Then, a silicone sheet was implanted between the flap and wound bed, and before flap transposition, a pullulan film was placed directly above this sheet. Four groups were assessed: DFO film, control film, DFO saline, and saline only. At day 2 the fourth side of the flap was cut, thereby isolating the skin flap from any original vasculature. Flap survival was quantified and harvested for histology. Neovascularization, ROS, and HIF stabilization were also assessed.

A novel drug delivery system was successfully created through the conjugation of DFO to pullulan. The DFO film proved to have consistent release of active drug in vivo. Flaps had decreased necrosis and increased viability and perfusion. A prolonged period of protection was evident compared with controls, which correlated strongly with HIF stabilization.

IPC may help to increase the success rate of flap transposition and decrease the complications associated with I/R injury. Delivery of targeted DFO is a practical method to enhance IPC to protect against tissue injury, prevent flap necrosis, increase neovascularization, and provide enhanced cellular tolerance to hypoxic and oxidative stress.



H. J. CHUN



M. G. GALVEZ

MONDAY
ROOM D-125

11:15 A.M.

A novel IL-10 signaling pathway in vascular smooth muscle cells modulates the acute p21^{Cip1}-mediated arterial wound response after vascular injury

ANGELA CATHERINE LEE, Research Scholar, Harvard Medical School

Preceptor: Manfred Boehm, M.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ Peripheral arterial disease (PAD) affects 8 million Americans and carries with it substantial morbidity and mortality. Invasive interventions for PAD have increasingly shifted from predominantly surgical to percutaneous endovascular procedures, including balloon angioplasty and stent placement. Despite this shift, amputation rates continue to increase and outcomes following lower extremity percutaneous interventions are inferior to those achieved in the coronary circulation. A major cause of sub-acute procedural failure following vascular intervention is intimal hyperplasia and restenosis due to vessel trauma sustained during stent placement/angioplasty. Our previous findings demonstrated that p21^{Cip1}, a cyclin-dependent kinase inhibitor, was upregulated in the setting of acute vascular injury, inhibiting vascular smooth muscle cell (VSMC) proliferation and subsequent intimal hyperplasia. However, the mechanism by which p21^{Cip1} is upregulated during acute vascular remodeling is unknown, and we hypothesized that IL-10, a potent anti-inflammatory cytokine, may be involved in this regulation.

To explore this, we stimulated mouse VSMCs with exogenous IL-10, resulting in increased p21^{Cip1} and p53 expression in a time-dependent manner, with increased protein levels of phosphorylated STAT3 also observed. This IL-10-mediated p21^{Cip1} upregulation was STAT3, p65, and p53 dependent, as shown by siRNA p65 and STAT3 knockdown and p53 knockout VSMCs. In vivo, p21^{Cip1} and p53 transcription peaked both at 6 hours and 7 days after femoral artery wire injury in control mice. However, in IL-10^{-/-} mice, p21^{Cip1} levels were unaffected at 6 hours while p53 expression was significantly decreased. At 7 days, during the period of neointimal formation, a 10- to 20-fold increase in p21^{Cip1} and a 3-fold increase in p53 were observed.

In conclusion, IL-10 upregulates p21^{Cip1} in mouse VSMCs in vitro in a STAT3-, p53-, and p65-dependent fashion. Furthermore, in the enhanced inflammatory physiology of IL-10^{-/-} mice, greater intimal hyperplasia was seen after acute vessel injury despite the significantly increased upregulation of p21^{Cip1}.



A. C. LEE



A. B. CONDREN

11:30 A.M.

In situ regulation of choroidal blood flow by smooth muscle cells and pericytes: an ex vivo confocal time-lapse imaging approach in sclerochoroidal explants

AUDREE B. CONDREN, Research Scholar, University of Oklahoma College of Medicine

Preceptors: Emily Y. Chew, M.D., and Wai T. Wong, M.D., Ph.D., National Eye Institute, National Institutes of Health

■ Investigations of choroidal blood vessel regulation have been largely performed in dissociated and fragmented tissue. Here, we aim to study the regulation of choroidal blood flow using an in situ model system of choroidal vessel behavior in intact sclerochoroidal explants using live imaging techniques.

Sclerochoroidal explants, containing intact sclera, choroid, and RPE, were acutely isolated from smooth muscle type α -actin (α SMA) promoter-driven green fluorescent protein (GFP) transgenic mice. Dynamic behavior of living smooth muscle cells and pericytes labeled with GFP was followed in situ using three-dimensional time-lapse confocal imaging in response to vasoactive agonists.

GFP-labeled, α SMA-positive perivascular cells had two distinct morphologies and distributions: 1) a band-like morphology that surrounds the circumference of vessels in a dense, ladder-like pattern and 2) a pericyte-like morphology with sprawling processes extending from a central soma that sparsely envelope choroidal vessels. Time-lapse imaging revealed that both cell morphologies exhibit dynamic structural changes that resulted in vessel constriction when vasoactive agonists were applied. α SMA-positive cells produced reversible vessel constriction in a dose-dependent manner to endothelin-1. Cellular behavior is also likely regulated by intracellular calcium as calcium entry, induced by A23187, a calcium-ionophore, produced vessel constriction, while calcium efflux, induced by transfer to a calcium-free medium, produced vessel dilation. The degree of vasoconstriction in imaged vessels increased as a function of increasing perivascular cell coverage, and is lower in vessels covered with low-density pericyte-like cells, compared to the vessels of similar diameter that are covered at a higher density by band-like cells.

Vital GFP-labeling of pericytes in transgenic mice presents a good in situ model (and a potential in vivo model) for studying regulatory changes in choroidal vasculature using live imaging. Perivascular α SMA-positive cells in the choroid are associated with distinct morphologies and distributions that may help confer functional diversity in the vasoregulation of the choroidal blood flow.

11:45 A.M.

Understanding energy production through the cell cycle: a synchronous yeast model system**MATTHEW J. REILLEY**, Research Scholar, The Warren Alpert Medical School of Brown University

Preceptor: Robert S. Balaban, Ph.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ Mitochondria are cell organelles that play an essential role in the production of energy using oxygen, a process known as aerobic respiration. While nearly all eukaryotic cells have mitochondria, some can exist in a state where they survive and reproduce in the absence of respiration. It is well established that rapidly dividing cell populations, such as many cancers, thrive in the absence of oxygen. Indeed, in 1966, Nobel laureate Otto Warburg proposed that the loss of mitochondrial function in cancer cells is centrally important to understanding cancer. Yet, 40 years later, the mechanisms underlying cellular control of mitochondrial respiration are not well understood. We hypothesize that there must be protein level changes in rapidly dividing cells that exist to regulate mitochondrial respiration. My goal for this year is to create a model of mitochondrial regulation in a rapidly dividing cell population.

We chose to work with the yeast *Schizosaccharomyces pombe* because it is a well-established model organism system and commonly used in cell-cycle research. A temperature-sensitive strain is cultured in liquid media at 25°C until cells are in the exponential growth phase. Cells are then incubated at 36°C to achieve cell-cycle synchrony before returning to the growth-permissive temperature. At intervals throughout the cell cycle, respiration measurements are taken using a Clark-type oxygen electrode, under control and uncoupled conditions. Additionally, cell counts are measured and protein samples are stored for later analysis.

Our initial results demonstrate that the respiration rate is variable during mitosis and stable during other parts of the cell cycle. Interestingly, coupled and uncoupled respiration rates remain proportional throughout the cell cycle, suggesting a potential coregulatory mechanism for total and available respiratory capacity. The results of proteomic analysis of the yeast at each time point will help to elucidate potential regulatory systems of mitochondrial respiration during mitosis.

Noon

The *Gne*^{M712T/M71T} hereditary inclusion body myopathy mouse model displays multiple glycoalyx alterations as part of a unique glomerulopathy**JUSTIN POLING**, Research Scholar, Vanderbilt University School of Medicine

Preceptors: William A. Gahl, M.D., Ph.D., and Marjan Huizing, Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Hereditary inclusion body myopathy (HIBM) is a rare, autosomal recessive, adult-onset progressive myopathy linked to mutations in the *Gne* gene, which encodes the rate-limiting enzyme in sialic acid biosynthesis. We created a double knockin mouse model of HIBM using the M712T mutation, which is the most common founder mutation in HIBM patients. Over 95% of homozygous mutant pups die before P3; upon necropsy, they display grossly hemorrhagic kidneys, while microscopy shows erythrocyte accumulation, podocyte effacement, and glomerular basement membrane splitting. We hypothesize that podocyte hyposialylation and resulting loss of negative charge disrupt the glomerular filtration apparatus by altering the podocyte glycoalyx. We also hypothesize that supplementing the diet with ManNAc, an intermediate sugar in the sialic acid biosynthesis pathway, will at least partially correct this phenotype.

Using the lectins HPA, WGA, PNA, LPA, and LFA for immunohistochemistry and Western blotting, glomeruli from homozygous mutant mice show hyposialylation as well as increased staining for β -galactose and GalNAc, which are sugars proximal to sialic acid on O-linked glycans. Hyposialylation also appears to mislocalize the sialoprotein podocalyxin only to the apical plasma membrane; we are currently confirming this finding with immuno-EM. Supplementation with ManNAc restores mutant pup survival to ~50%; using the above techniques, we are investigating whether this treatment also restores the glomerular defects.

While ManNAc might be a promising therapy for human HIBM patients, our results from lectin stains indicate that glycoalyx alterations might also contribute to a number of human nephropathies. We are currently comparing renal biopsies from control patients to those of patients with renal diseases, including minimal change disease, focal segmental glomerulosclerosis, lupus nephropathy, and HIV nephropathy to determine whether glycoalyx alterations are found in any of these conditions.



M. J. REILLEY



J. POLING

MONDAY
ROOM D-125

12:15 P.M.

Role of galectin-3 on the development of pulmonary fibrosis in Hermansky-Pudlak syndrome type I

CAROLINE YEAGER, Research Scholar,
Duke University School of Medicine

Preceptors: William A. Gahl, M.D., Ph.D., and Bernadette R. Gochuico, M.D., National Human Genome Research Institute, National Institutes of Health

■ Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder that results in the malformation and mistrafficking of lysosome-related organelles (LROs). Although rare in the general population, in northwest Puerto Rico the estimated prevalence of HPS is greater than that of cystic fibrosis in the United States. The melanosomes in melanocytes and the delta granules in platelets are two specific LROs, and their malfunction leads to two of the key clinical manifestations of the disorder: oculocutaneous hypopigmentation and a bleeding diathesis. HPS-1 and HPS-4, two subtypes of HPS that both affect the biogenesis of lysosome-related organelles complex-3 (BLOC-3) protein complex, also develop pulmonary fibrosis. Pulmonary fibrosis is in fact the

leading cause of mortality in HPS-1 patients, and all known patients with HPS-1 show evidence of pulmonary fibrosis by age 60 years. Currently, the only treatments available for pulmonary fibrosis are experimental drug trials and lung transplantation.

How HPS-1 and HPS-4 cause interstitial lung disease is unknown. Of particular interest is the type II pneumocyte, which stores surfactant protein in lamellar bodies, a LRO. Human lung specimens from HPS-1 patients with pulmonary fibrosis do show a distinctive histology with excessive giant lamellar bodies in proliferating type II pneumocytes and ceroid lipofuscin deposition in alveolar macrophages. We hypothesize that a lectin produced by type II pneumocytes, galectin-3, plays a key role in the development of pulmonary fibrosis in patients with HPS-1. Through immunohistochemistry staining, we have shown that galectin-3 is present in the type II pneumocytes and alveolar macrophages of the HPS-1 lung at increased levels when compared to samples from patients with idiopathic pulmonary fibrosis (IPF) and normal volunteers. In addition, we have found elevated galectin-3 levels in the bronchoalveolar lavage fluid (BALF) of HPS-1 patients with pulmonary fibrosis. Currently, we are examining the role of galectin-3 stimulation on the function of fibroblasts.



C. YEAGER

9:00 A.M.

Analysis of the cell of origin of lung adenocarcinoma**MARK ONAITIS, M.D.**, Early Career Awardee, Duke University School of Medicine

■ The cell(s)-of-origin of lung adenocarcinoma are currently unknown. We have used inducible Cre recombinase mouse models to express oncogenic K-Ras in specific bronchial epithelial cells. We isolated RNA both from adenocarcinomas that arose from K-Ras induction in Clara cells and from Clara cells themselves and performed microarrays. A K-Ras-Clara cell expression signature was developed and applied to a cohort of resected human adenocarcinomas.

Lox-stop-lox K-Ras^{G12D} mice were crossed with mice expressing Clara cell antigen 10 (CC10) Cre-estrogen receptor (CreER) and with Keratin 5 (K5) CreER mice. After weaning, tamoxifen was injected to activate Cre in either Clara cells (CC10-CreER) or basal cells (K5-CreER). Adenomas and then adenocarcinomas formed in the K-Ras^{G12D}-CC10-CreER mice. The tumors clustered about the bronchoalveolar duct junction despite Cre expression in Clara cells throughout the airway. By 21 weeks, hyperplastic changes were seen in more proximal bronchi in several mice. No bronchial phenotype occurred in the K-Ras^{G12D}-K5-CreER mice. RNA isolation/microarray analysis was performed on these adenocarcinomas and normal Clara cells. Probes significantly differently expressed between the Clara cells and the K-Ras-positive tumors were identified and applied to a cohort of human adenocarcinoma samples. Those human tumors with expression profiles most similar to that of the K-Ras tumors exhibited a significantly worse prognosis than those with dissimilar profiles.

In conclusion, expression of oncogenic K-Ras in the Clara cells of adult mice leads to adenocarcinoma that seems to arise at the bronchoalveolar duct junction. A subset of CC10-positive cells in this location is vulnerable to K-Ras-induced transformation. Whether this phenomenon is secondary to local environment or to cell-intrinsic factors requires further investigation. These tumors may prove useful for therapeutic development in that a similar subset of human adenocarcinomas may be identified by expression analysis.

9:15 A.M.

Toward the functional validation of BRCA1-Associated RING Domain 1 as a neuroblastoma predisposition gene**KRISTOPHER BOSSE**, Medical Fellow, University of Pennsylvania School of Medicine

Mentor: John M. Maris, M.D., University of Pennsylvania School of Medicine, The Children's Hospital of Philadelphia

■ Neuroblastoma is a common and often lethal pediatric malignancy. We hypothesize that sporadic neuroblastoma is a polygenic disease and that susceptibility is conferred by common variation at many genetic loci. Using a genome-wide association study approach, we identified a highly significant and robustly replicated association of the high-risk subset of neuroblastoma with six SNPs at 2q35 within the *BRCA1-Associated RING Domain 1* (*BARD1*) gene ($P = 8.65 \times 10^{-18}$ – 8.04×10^{-12} , OR = 1.56–1.68; Capasso et al., *Nature Genetics*, in press). The goal of this study is to identify the disease causal variants at 2q35 and determine how these contribute to neuroblastoma tumorigenesis.

We have fine mapped 2q35 with HapMap CEU data and found that these six SNPs are all in strong linkage with 14 genomic regions densely occupied with regulatory domains, suggesting that common variation at 2q35 may alter *BARD1* expression. We next studied *BARD1* expression in neuroblastoma cell lines ($n = 20$), all derived from high-risk cases. *BARD1* mRNA expression was increased in cell lines with a homozygous risk allele genotype ($P = 0.02$). In contrast, expression of the major *BARD1* protein isoform was decreased in neuroblastoma cell lines with a predominant risk allele haplotype. To date, 15 alternatively spliced *BARD1* transcripts have been identified. Our initial results suggest that a subset of these transcripts may be more abundant in cell lines with a predominant risk allele haplotype, possibly explaining the discrepancy between *BARD1* mRNA and protein expression trends.

We have shown that common variation at 2q35 correlates with alterations in *BARD1* expression. We will next identify all genetic variants at 2q35 via regional next-generation resequencing and compare these data with *BARD1* expression patterns. In addition, to determine whether *BARD1* acts as a neuroblastoma suppressor gene, we are studying its overexpression in cell line models.

MONDAY
AUDITORIUM

M. ONAITIS



K. BOSSE

MONDAY
AUDITORIUM

9:30 A.M.

γ -Interferon-mediated superinduction of B7-H1 in PTEN-deficient glioma patients: an immunoresistant phenotype that can confound response to cancer vaccine therapy

SEUNGGU J. HAN, Medical Fellow, University of California, San Francisco, School of Medicine

Mentor: Andrew T. Parsa, M.D., Ph.D., University of California, San Francisco, School of Medicine

■ Malignant glioma is a terminal diagnosis, and standard therapies are associated with significant toxicities. Immunotherapy can target glioma cells without damaging healthy tissue. Challenges to successful immunotherapy include identifying an effective vaccine source and overcoming glioma immunoresistance. Here, we describe a uniquely immunoresistant phenotype within the context of an ongoing phase I/II glioma vaccine trial.

Recurrent glioma patients were enrolled in an open label single arm phase I/II study with autologous heat shock protein (HSP) vaccine, containing 25 μ g of gp96 glycoprotein isolated from autologous glioma tissue taken at time of surgery. Immunomonitoring of trial patients consisted of isolation of peripheral blood lymphocytes (PBLs) prior to tumor resection and at each vaccination. PBLs were restimulated with autologous vaccine and evaluated for cytokine production, including γ -interferon. Primary glioma cell cultures were established and characterized with respect to PTEN phenotype by immunohistochemical staining. Levels of B7-H1 protein, a potentially immunosuppressive ligand, were evaluated before and after treatment with γ -interferon.

Phase I results of the ongoing trial confirmed that autologous HSP vaccine is safe and well tolerated in recurrent glioma patients. Immunomonitoring showed a clear natural killer cell response and tumor-specific T cell response characterized by γ -interferon secretion upon restimulation in all patients. As shown previously, PTEN-deficient glioma cells have a significantly greater level of B7-H1 expression, compared to PTEN wild types. γ -Interferon treatment of PTEN-deficient cells induced more than a 10-fold increase in B7-H1 expression compared to PTEN wild-type controls. Consistent with these findings, interim analysis shows a trend toward greater survival after vaccination in patients who are PTEN wild type, compared to PTEN deficient.

Loss of the tumor-suppressor gene PTEN in glioma results in upregulation of the immunosuppressive protein B7-H1. Exposure of tumor cells to γ -interferon causes superinduction of B7-H1, defining a subset of patients who may be especially resistant to glioma vaccine therapy.



S. J. HAN



Z. ZUMSTEG

9:45 A.M.

Targeting the 26S proteasome for radiotherapeutic benefit in glioblastoma multiforme

ZACHARY ZUMSTEG, Medical Fellow, David Geffen School of Medicine at UCLA

Mentor: William McBride, D.Sc., David Geffen School of Medicine at UCLA

■ Despite increasing insight into the molecular pathogenesis of glioblastoma multiforme (GBM), this malignancy remains highly resistant to current frontline treatment modalities, including radiation. Previously, our lab has shown that irradiation of many cell lines rapidly inhibits all proteolytic activities of the 26S proteasome, and that molecular proteasome inhibitors radiosensitize certain cancer cells. Thus, we hypothesized that proteasome-inhibiting molecules may radiosensitize GBM cells expressing the epidermal growth factor receptor variant III (EGFRvIII), which are uniquely resistant to radiation-induced proteasome inhibition. Using a high-throughput screening (HTS) approach, we sought to identify novel proteasome inhibitors in GBM.

We engineered U87EGFRvIII cells to stably express a fusion protein of luciferase and the carboxy-terminal degron of ornithine decarboxylase, which is recognized and degraded by the proteasome in a ubiquitin-independent manner. These cells were used for HTS of more than 60,000 compounds for proteasome-inhibiting potential. Cells were plated, treated with each compound at 10 μ M, and then the luminescence was measured. Several novel classes of proteasome inhibitors were identified with this approach, including the isoflavone family, numerous tyrosine kinase inhibitors, and the thiazole antibiotics. Thiostrepton, a member of this last class, was chosen for further characterization, as it caused by far the greatest increase in luminescence of any bioactive molecule. A fluorogenic assay showed that thiostrepton directly inhibited the chymotrypsin-like and caspase-like, but not trypsin-like, proteolytic activity of the 26S proteasome in GBM cell lines. In addition, thiostrepton caused a dose-dependent decrease in the viability of U87EGFRvIII cells.

Thiostrepton has recently been described as an inhibitor of the forkhead box M1 (FoxM1) transcription factor. FoxM1, which is overexpressed in GBM, critically regulates cell cycle progression, mitosis, and DNA repair. We are currently investigating if other known proteasome inhibitors also inhibit FoxM1. In addition, clonogenic radiosensitization assays and animal studies with thiostrepton are underway.

10:00 A.M.

The role of cell cycle in epidermal growth factor receptor-mediated radiosensitization**SUSAN M. HINIKER**, Medical Fellow, University of Michigan Medical School

Mentor: Theodore S. Lawrence, M.D., Ph.D., University of Michigan Medical School

■ Sensitivity of tumor cells to radiation therapy is a critical determinant of outcome in the treatment of many cancers. Recent advances in the field of signal transduction have suggested a key role for the epidermal growth factor receptor (EGFR) in radiation sensitivity; therefore, EGFR inhibitors are now combined with radiotherapy in the treatment of various cancers. Because both radiation and EGFR inhibition can induce cell cycle effects, the cell cycle deserves careful consideration in the optimization of treatment combining EGFR inhibitors and radiation. We sought to determine the effects of radiation on both quiescent cells in $G_{0/1}$ and proliferating cells in culture and found that quiescent cells are significantly more sensitive to ionizing radiation than proliferating cells. In order to understand this unexpected difference in radiosensitivity, we analyzed the effects of radiation on EGFR activation and the cell cycle. We found that upon irradiation, the EGFR and ERK pathway was activated in quiescent cells, leading to progression of cells from G_1 to S, but this activation and progression did not occur in the proliferating cells. Inhibition of this activation blocked S-phase progression and protected quiescent cells from radiation-induced death, with the effects particularly pronounced for EGFR inhibition. In a control system using CHO cells, which lack EGFR expression, exogenously expressed EGFR also became activated after radiation in quiescent cells, but not proliferating cells. Moreover, quiescent cells expressing EGFR underwent increased radiation-induced cell death as compared to both proliferating CHO cells expressing EGFR and quiescent wild-type CHO cells. Collectively, our data demonstrate a novel phenomenon of radiation-induced enhancement of cell death in quiescent cells involving the EGFR and ERK pathways. This research may have important implications for the rational design of clinical trials optimizing treatment schedule in the combination of EGFR inhibitors with chemotherapy and radiation therapy.

10:15 A.M.

Effect of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) inhibition on tumor oxygenation, interstitial fluid pressure, and liposome delivery**TINA D. TAILOR**, Medical Fellow, Duke University School of Medicine

Mentor: Mark W. Dewhirst, D.V.M., Ph.D., Duke University

■ Elevated interstitial fluid pressure (IFP) and hypoxia are hallmarks of solid tumors that pose an inherent barrier to chemotherapy and radiotherapy. While the relationships between these two factors and their underlying mechanisms are not fully elucidated, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are two tyrosine kinases that are thought to play a causal role. VEGF, a principle pro-angiogenic factor, drives aberrant vessel sprouting, as well as endothelial hyperpermeability. The resultant vascular network is heterogeneously distributed and unable to maintain transvascular pressure gradients, making it inadequate for oxygen/nutrient delivery and drug transport. Intratumor transport is further limited by a dense stroma, which is regulated by PDGF-mediated recruitment of fibroblasts, pericytes, and other extracellular matrix (ECM) components. The solid stress of this ECM compromises blood flow, contributes to IFP elevation, and impedes the distribution of chemotherapeutics, particularly macromolecular agents, through the tumor interstitium.

We hypothesize that targeted inhibition of VEGF and PDGF will reduce IFP, enhance perfusion via normalization of vessel/stromal architecture, and improve delivery of macromolecular agents. Consistent with the aforementioned theories of hypoxia and IFP, fluorescent microscopy reveals that Doxil (liposomal Doxorubicin) accumulation is limited solely to perivascular regions in nude mice bearing human non-small-cell lung cancer xenografts. After eight days of treatment with an anti-VEGF/anti-PDGF inhibitor, tumors exhibit a significant reduction in IFP. Spectroscopic measurements of hemoglobin saturation reveal a peak in oxygenation after this same treatment course. Immunohistochemical staining for CD31 (endothelial marker) in excised tumors reveals a decrease in microvessel density. These data suggest that dropout of abnormal vasculature and IFP reduction may improve perfusion.

We conclude that dual inhibition of VEGF and PDGF leads to a window of improved oxygenation and decreased IFP. Adjuvant radiotherapy and chemotherapy delivered during this window period may exhibit enhanced antitumor effect.



S. M. HINIKER



T. D. TAILOR

MONDAY
AUDITORIUM

10:45 A.M.

MicroRNA-7 is a potential tumor suppressor inhibiting oncogenic pathways in gliomas

BENJAMIN PUROW, M.D., Early Career Awardee, University of Virginia School of Medicine



B. PUROW

■ Gliomas are the most common and lethal brain tumors and are highly resistant to standard radiation and chemotherapy. While ongoing research has shown the importance of pathways such as EGFR/Ras and Akt in gliomagenesis, it is essential in developing new therapies that we better understand the molecular underpinnings of these cancers. Recent reports have suggested that many cancers, including gliomas, are strongly influenced by a newly discovered class of noncoding RNAs termed microRNAs, short RNAs that each suppress expression of many target genes through binding to the 3' untranslated region (3'-UTR). Several microRNAs have been found to be up- or downregulated in various cancers, but little has been published on their roles in controlling key oncogenic pathways. Our previous work on the Notch pathway and the EGF receptor in gliomas led us to observe target sites for microRNA-7 (miR-7), a microRNA previously noted to target Notch mediators in *Drosophila*, in the 3'-UTR of human EGFR, an important oncogene in gliomas. We have confirmed that miR-7 potently regulates EGFR expression in human cells. Furthermore, bioinformatics searches predicted that miR-7 would target members of the Notch and Akt pathways, also important in glioma cell survival. Our preliminary experiments have supported inhibition of these pathways by miR-7 in glioma cells in vitro. We also demonstrated down-regulated expression of mature miR-7 in human glioma samples versus surrounding brain through a mechanism involving decreased processing. Importantly, transfection of miR-7 caused apoptosis and inhibited invasion of human glioblastoma lines in vitro. These results identify miR-7 as a potential tumor suppressor that regulates major oncogenic pathways in gliomas and suggest its delivery as a therapy for these cancers.



H. A. ZAIDI

11:00 A.M.

Adipose-derived mesenchymal stem cells represent a novel delivery vehicle for therapeutic agents in the treatment of intracranial gliomas

HASAN A. ZAIDI, Medical Fellow, Johns Hopkins University School of Medicine

Mentor: Alfredo Quiñones-Hinojosa, M.D., Johns Hopkins University School of Medicine

■ Despite a multidisciplinary approach, the prognosis for patients with intracranial glioma remains poor. Current therapeutic modalities are nonspecific and fail to target local intraparenchymal spread of malignant brain tumor stem cells. Mesenchymal stem cells isolated from bone marrow (BM-MSCs) show promise in being able to home in and target malignant cells of various types while effectively delivering therapeutic agents to the site of pathology. However, concerns over dangers in harvesting tissue for cell expansion and the potential of BM-MSCs to integrate into the tumor parenchyma have limited their clinical use. Mesenchymal stem cells from adipose tissue (AMSC) can be harvested safely from patients and express many of the same cell surface markers as BM-MSCs. Here, we demonstrate that AMSCs selectively migrate toward intracranial gliomas in immunocompetent mice and reduce the growth and proliferation of tumor cells both in vitro and in vivo. When genetically modified using a retroviral vector to express interleukin-12, AMSCs enhance survival of mice engrafted with malignant gliomas. Thus, adipose tissue represents a safe and easily accessible source of MSCs, which can be used as a delivery vehicle in the treatment of intracranial gliomas.

11:15 A.M.

The effects of PDGFR- α stimulation on neural and brain tumor stem cell behavior and molecular signaling**THOMAS ADAM KOSZTOWSKI**, Medical Fellow, Johns Hopkins University School of Medicine

Mentors: Alfredo Quiñones-Hinojosa, M.D., and Hongjun Song, Ph.D., Johns Hopkins University School of Medicine

■ A subpopulation of malignant glioma cells with increased resistance to chemo- and radiotherapy, termed brain tumor stem cells (BTSCs), offers a potential cell of origin for glioblastoma multiforme (GBM). PDGFR (platelet-derived growth factor receptor) mutation is one of the most frequently found mutations in GBM tumors, especially secondary. RT-PCR demonstrated markedly increased levels of PDGFR- α in both primary and secondary human GBMs relative to normal human astrocytes and neural stem cells (NSCs). MTT assays showed significantly increased proliferation in a subset of these GBM cell lines upon stimulation of PDGFR- α in both astrocytic and neurosphere cell populations. Clonal neurosphere assays demonstrated increased neurosphere formation and increased BTSC proliferation in PDGF-AA conditions. These clonal neurosphere assays were followed up by differentiation assays to see whether growth in PDGF-AA conditions increased differentiation of these BTSCs into the oligodendrocyte lineage; however, these BTSCs had a tendency to simultaneously express cell markers from several different cell lineages. We are using RT-PCR and Western blots to better assess whether or not PDGFR- α stimulation has any effect on coaxing these BTSCs down any specific lineage pathway. In vivo experiments have shown that pretreatment of BTSCs in PDGF-AA conditions was associated with larger tumors on MRI. Stimulation of PDGFR- α also increases the migration and invasion of BTSCs. Western blots have demonstrated that PDGFR- α is indeed activated in these BTSCs and that activation of Akt and MAPK occurs downstream. PDGFR- α has been knocked down with shRNA, and further characterization of the downstream signaling effects of the receptor in the proliferative and migratory signaling pathways is underway. Human NSCs with upregulated levels of PDGFR- α have been created to determine whether the PDGF autocrine loop is sufficient to transform human NSCs into BTSCs. All these studies will help us better understand the origin and biology of brain tumors so that patient care may be improved.

11:30 A.M.

Investigating the therapeutic value of Wnt/ β -catenin activation in malignant melanoma**CORINNE TARASKA**, Medical Fellow, University of Washington School of Medicine

Mentors: Andy J. Chien, M.D., Ph.D., and Randall T. Moon, Ph.D., University of Washington School of Medicine

■ Over the past 30 years, no improvement has occurred in survival rates of patients with malignant melanoma. Prior research implicates the Wnt/ β -catenin signaling pathway in cancer progression. Mutations causing constitutive activation of the Wnt pathway are known to potentiate some cancers, notably colorectal cancer; however, our recent data oppose this traditional understanding in the context of melanoma. Specifically, melanomas with elevated levels of nuclear β -catenin, a surrogate marker for Wnt activation, correlate with improved prognosis. This supports the hypothesis that activation of the Wnt/ β -catenin pathway is beneficial in malignant melanoma.

A high-throughput cell-based screen of 30,000 small molecules was conducted utilizing a β -catenin activating reporter (BAR) to identify compounds that activate Wnt signaling independently or in synergy with Wnt ligand. Established human melanoma lines were transduced with virus encoding BAR and then used to validate the top 21 screen hits via flow cytometry. PCR analysis of downstream Wnt targets and known melanoma-related genes (*KIT*, *MITF*, *AXIN2*, and *TRPM1*) was also conducted for further validation of these compounds as true activators of Wnt/ β -catenin signaling. The proliferative and cell cycle effects of these small molecules and Wnt-conditioned media were tested via MTT assay and flow cytometry, respectively, on human melanoma lines.

Wnt activation in human melanoma lines was validated for 13 small molecules, including several FDA-approved compounds. MTT assays and flow cytometry revealed marked reduction in proliferation and alteration of cell cycle in response to select compounds.

Continuing PCR and Western blot analysis investigates the transcriptional and translational effects of Wnt ligand and small-molecule Wnt activators on human melanoma lines. Compounds with robust Wnt activity will undergo murine in vivo testing via B16 murine melanoma tumor grafts in immunocompetent hosts and human melanoma xenografts in immunocompromised murine hosts for effects on metastasis.



T. A. KOSZTOWSKI



C. TARASKA

MONDAY
AUDITORIUM

11:45 A.M.

Engineering a tumor-specific, apoptosis-resistant T cell for adoptive cell transfer therapy

ANUSHA KALBASI, Research Scholar, David Geffen School of Medicine at UCLA

Steven A. Rosenberg, M.D., Ph.D., National Cancer Institute, National Institutes of Health

■ Adoptive cell transfer followed by IL-2 administration is an effective antitumor therapy in patients with advanced melanoma. Persistence of T cells after adoptive transfer correlates with antitumor response and may be important for improved tumor treatment. In vivo withdrawal of concomitant IL-2 therapy, activation-induced T cell death, and other pro-apoptotic conditions created locally and systemically by the tumor may limit T cell persistence. Recently, T cell receptor (TCR) gene modified lymphocytes have been used in adoptive immunotherapy. In an attempt to improve persistence of these lymphocytes, we used retroviruses expressing Bcl-2 or Bcl-xL, anti-apoptotic genes of the Bcl2 family, and the MART-1 melanoma tumor antigen specific TCR DMF5 to cotransduce human peripheral blood lymphocytes (PBLs). We tested the ability of Bcl-2 or Bcl-xL to improve the growth, survival, phenotype, and tumor-specific function of DMF5-transduced lymphocytes.

In contrast with DMF5 TCR alone, lymphocytes cotransduced with DMF5 TCR and Bcl-2 or Bcl-xL demonstrated 1.14 ± 0.52 and 0.72 ± 0.30 fold growth or survival in vitro, respectively, 16 days following IL-2 withdrawal. After four days of IL-2 withdrawal, DMF5/Bcl-2 and DMF5/Bcl-xL cotransduced lymphocytes demonstrated 3.7 ± 1.2 and 4.9 ± 2 fold increase in survival, respectively, in comparison to DMF5-transduced lymphocytes, while producing similar levels of IFN- γ per cell following tumor stimulation, as measured by ELISA. Cotransduction did not alter the phenotype of lymphocytes with respect to a panel of T cell differentiation markers. Finally, whereas coculture of DMF5-transduced lymphocytes with melanoma tumors expressing cognate antigen induces apoptosis of lymphocytes as measured by annexinV, cotransduction with Bcl-2 inhibits this apoptosis. Therefore, by coexpressing Bcl-2 or Bcl-xL with a tumor-specific TCR, we have engineered a lymphocyte that resists many of the putative pro-apoptotic in vivo conditions of adoptive transfer without altering its tumor-specific function or phenotype, and thus may demonstrate improved antitumor effectiveness in vivo following cell transfer.



A. KALBASI



M. J. GOLDSTEIN

Noon

Anticancer CD4 memory T cells: identification by CD44 and CD137

MATTHEW J. GOLDSTEIN, Medical Fellow, Stanford University School of Medicine

Mentor: Ron Levy, M.D., Stanford University School of Medicine

■ A goal of cancer immunotherapy is an adaptive immune response against cancer-specific antigens. The memory T cell is a central mediator of this response and can give rise to effector cells as well as self-renew. Traditionally, cytotoxic CD8 T cells (CTLs) have been described as the primary mediators of anticancer immune responses. In adoptive immunotherapy studies, a specific subpopulation of memory CD8⁺ T cells—central memory (T_{CM}) T cells (CD44^{hi}CD62L^{hi})—has prolonged persistence (Berger, 2008) and superior anticancer efficacy (Klebanoff, 2005) over other subpopulations. Accordingly, many immunotherapy strategies have focused on enhancing this population.

Here, we describe anticancer immunity mediated exclusively by CD4⁺ T cells. We show that vaccine-primed CD4⁺ but not CD8⁺ T cells prevent tumor growth when adoptively transferred into irradiated, syngeneic, C57BL/6 recipient mice. Transferred CD4⁺ cells provided long-term immunity and could be recovered from recipient mice over 100 days after transfer. In vitro assays 10 days after transfer confirmed that CD4⁺ but not CD8⁺ T cells produce IFN- γ in response to co-culture with tumor and demonstrated that IFN- γ ⁺ cells cluster in the CD44^{hi} population. Further characterization of this population showed that >94% of CD44^{hi} CD4⁺ T cells expressed an effector phenotype (CD62L^{lo} and CCR7^{lo}). Interestingly, cells recovered from 100-day-old mice have diverse CD62L and CCR7 surface marker profiles, suggesting that several “types” of anticancer CD4⁺ T cells arise and persist as memory populations.

In conclusion, we found that T regulatory cells (T_{reg}) (CD25⁺FoxP3⁺) are present in the CD4⁺CD44^{hi} population of vaccinated donor mice. We demonstrate that these T_{reg} cells express CD137. Adoptive transfer of <120,000 CD44^{hi}CD137⁻ but not CD44^{hi}CD137⁺ CD4⁺ T cells provided significant anticancer immunity. These results support the enrichment of a CD4⁺ anticancer immunological progenitor cell within the CD44^{hi}CD137⁻ population.

12:15 P.M.

CD47 is an independent prognostic factor and therapeutic antibody target on acute myeloid leukemia stem cells**MARK P. CHAO**, Medical Fellow, Stanford University School of Medicine

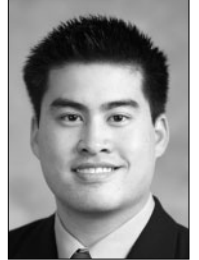
Mentor: Irving L. Weissman M.D., Stanford University School of Medicine

■ Acute myeloid leukemia (AML) is a clonal malignancy with poor long-term survival. A permanent cure for AML requires elimination of leukemia stem cells (LSCs), the only cell type capable of initiating and maintaining leukemic disease. This can be achieved by targeting antigens that have unique expression profiles on LSCs compared to normal hematopoietic stem cells (HSCs).

Based on cell surface expression studies, we have identified CD47 as a cell surface marker with increased expression on LSCs compared to HSCs. A major function of CD47 is to inhibit the immune

phagocytic response of host phagocytes, a function that is an attractive mechanism for cancer immunoevasion. We therefore hypothesized that increased CD47 expression on AML LSCs contributes to pathogenesis by inhibiting their phagocytosis through the interaction of CD47 with an inhibitory receptor on phagocytes, Sirp- α . We found that CD47 was more highly expressed on AML LSCs than their normal counterparts, and that increased CD47 expression predicted worse overall survival in three independent cohorts of adult AML patients. Furthermore, blocking monoclonal antibodies directed against CD47 preferentially enabled phagocytosis of AML LSCs and inhibited their engraftment in vivo. Finally, treatment of human AML LSC-engrafted mice with an anti-CD47 antibody eliminated AML and targeted AML LSCs.

In summary, CD47 is an independent prognostic factor in AML and can be therapeutically targeted by a monoclonal blocking antibody for elimination of leukemic disease.



M. P. CHAO

MONDAY
AUDITORIUM

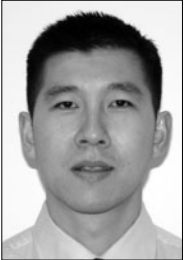
1:30 P.M.

Different by destruction: unequal inheritance of the transcription factor T-bet as a mechanism to diversify daughter T cell fates

JOHN T. CHANG, M.D., Early Career Awardee, University of Pennsylvania School of Medicine

■ During an infection with a microbial pathogen, antigen-specific T cells must give rise to differentiated effector cells for acute defense as well as long-lived memory cells capable of self-renewal. Asymmetric cell division might represent a mechanism to ensure that appropriate diversity of cell fate arises from the descendants of a single lymphocyte during an immune response. During asymmetric cell division, protein determinants are segregated to one side of the plane of division; the unequal inheritance of critical proteins results in distinct fates for the daughter cells. We have recently shown that a naïve T cell, activated by a microbial pathogen *in vivo*, divides asymmetrically, resulting in one daughter that appears to become an effector cell and the other that appears to become a memory cell. However, it remains unknown what fate determinants, if inherited unequally by the daughter cells, might specify these distinct lineages.

We now show that the fate-determining transcription factor T-bet is asymmetrically inherited by dividing T cells recruited into an immune response. T-bet is induced in interphase T cells within hours of activation. During mitosis, T-bet undergoes proteasome-dependent degradation. Mitotic destruction is mediated by T cell receptor-induced tyrosine phosphorylation of T-bet. Unequal inheritance of T-bet is associated with asymmetric segregation of the proteasomal degradative machinery during mitosis and cytokinesis. Mutations of T-bet at the critical tyrosine and those disabling the T cell receptor-associated kinase, ITK, both result in symmetric inheritance of T-bet without affecting asymmetry of the proteasome. These results suggest that two experimentally distinct mechanisms promote the unequal inheritance of T-bet by initial daughter T cells: one signal that targets T-bet for mitotic destruction and another signal that renders inequality in the inheritance of the cellular machinery that destroys T-bet. These findings offer a new framework for understanding how signaling to a single T lymphocyte can result in unequal fate determination of its daughter cells.



J. T. CHANG



Y. R. CHAN

1:45 P.M.

Lipocalin 2 is required for pulmonary host defense against *Klebsiella* infection

YVONNE R. CHAN, M.D., Early Career Awardee, University of Pittsburgh School of Medicine

■ Antimicrobial proteins comprise a significant component of the acute innate immune response to infection. They are induced by pattern recognition receptors as well as by cytokines of the innate and adaptive immune pathways and play important roles in infection control and immunomodulatory homeostasis. Lipocalin 2 (siderocalin, NGAL, 24p3), a siderophore-binding antimicrobial protein, is critical for control of systemic infection with *Escherichia coli*; however, its role in mucosal immunity in the respiratory tract is unknown.

In the current study, we examined the role of lipocalin 2 in pulmonary defense against bacterial infection and the mechanism of its regulation in a mouse model of *Klebsiella pneumoniae* infection. We demonstrate that recombinant lipocalin 2 has *in vitro* activity against *K. pneumoniae*. *In vivo*, we found that this protein is robustly upregulated by TLR4- and IL-1 β -dependent pathways by examining its upregulation in various cytokine knockout models.

In this study, we found that lipocalin 2 is rapidly and robustly induced by *K. pneumoniae* infection and is TLR4 dependent. IL-1 β and IL-17 also individually induce lipocalin 2. Mucosal administration of IL-1 β alone could reconstitute the lipocalin 2 deficiency in TLR4 knockout animals and rescue them from infection. Lipocalin 2-deficient animals have impaired lung bacterial clearance in this model, and mucosal reconstitution of lipocalin 2 protein in these animals resulted in rescue of this phenotype. We conclude that lipocalin 2 is a crucial component of mucosal immune defense against pulmonary infection with *K. pneumoniae*.

2:00 P.M.

Functional genomic analysis of *Caenorhabditis elegans* innate immunity**COSTI SIFRI, M.D.**, Early Career Awardee,
University of Virginia Health Sciences Center

■ Innate immune responses are evolutionarily conserved across phylogeny and are essential for the early recognition of and defense against invading microorganisms. We have shown that the nematode *Caenorhabditis elegans* is susceptible to lethal infection by the Gram-positive human pathogen *Staphylococcus aureus*. Our central hypothesis is that *C. elegans* can be used to identify evolutionarily conserved innate immune responses to *S. aureus* infection. Toward that goal, we are using systematic genome-wide RNA inhibition to screen for *C. elegans* determinants that alter susceptibility to *S. aureus* infection.

After screening more than 3,200 targets (16.8% of all *C. elegans* genes), we have identified 290 (9.0%) candidates that increase susceptibility (enhanced susceptibility to staphylococci, *ess*) and 122 (3.8%) candidates that reduce susceptibility (enhanced resistance to staphylococci, *ers*) to *S. aureus* infection. While few *ers* targets appear to play a direct role in defense responses, *ess* targets are enriched in genes that are or can be hypothesized to be involved in innate immunity. Examples of possible effector products include C-type lectins, CUB domain proteins, and metridin-like ShK toxins. Several candidates may be involved in innate immune signaling, including genes of the IGF-1 and mitogen-activated protein signaling pathways, genes encoding products with leucine-rich repeats, and nuclear hormone receptor genes. Finally, a number of candidates have previously been shown to be differentially expressed when exposed to pathogens in microarray analysis, many of which encode proteins of unknown function.

We are in the process of performing hit validation, epistasis analysis, and detailed morphologic assessment of the silenced nematodes. Our expectation is that these analyses will identify a large number of new candidate immunity genes of varying biological function and that some candidates will belong to functional classes or pathways not previously implicated in innate immunity.

2:15 P.M.

Natural killer cell microRNA transcriptome defined by massively parallel sequencing**TODD A. FEHNIGER, M.D., PH.D.**, Early Career Awardee, Washington University School of Medicine

■ MicroRNAs serve as critical small RNA regulators of T and B lymphocyte development and function; however, their expression profile and role in natural killer (NK) cell biology is unknown. To ask informed questions about the role of specific microRNAs in the regulation of NK cells, elucidation of the microRNAs expressed in NK cells is required. To define the NK cell microRNA transcriptome, we adapted “next-generation” sequencing technology. Two cDNA libraries, representing 18–26 bp small RNAs from resting and IL-15-activated murine splenic NK cells ($\geq 99\%$ NK1.1⁺CD3⁻), were sequenced on Illumina’s genome analyzer. From these libraries, 8,790,618 (4,678,780 resting NK and 4,111,838 activated NK) raw reads were generated, with identification of 3,902,309 (2,038,243 resting NK and 1,864,066 activated NK) high-quality reads following filtering steps for sequence accuracy and base calling quality. Known microRNA frequencies were determined by BLAST alignments to mature/mature* microRNA sequences from miRBase v12, and 1,944,830 (50%) of NK cell sequence reads represented 372 of 631 known microRNAs. The five most abundant microRNAs in NK cells were miR-21, miR-16, miR-142-3p, miR-142-5p, and miR-24. Notably, multiple variants of each microRNA (isomiRs) were observed, revealing an underlying complexity to the NK microRNA transcriptome. To discover novel microRNAs, sequence reads were aligned to the mouse genome, and putative novel microRNA genes were identified by the read coverage and predicted folding characteristics of alignment “clusters.” Analysis of undefined small RNA sequences is ongoing, and so far, 11 candidate novel microRNA genes have been identified in the top 100 expressed “clusters” in NK cells. These data identify the resting and IL-15-activated NK cell microRNA transcriptomes, which provide an important framework to investigate the role of microRNAs in NK cell development and function.



C. SIFRI



T. A. FEHNIGER

MONDAY
ATRIUM

POSTER A1

A mineralocorticoid receptor trans-repression pathway suppresses endothelial inflammation

KEVIN P. BLAINE, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Preceptor: Robert L. Danner, M.D., National Institutes of Health Clinical Center, Critical Care Medicine Department

■ Septic shock is a hyperinflammatory state caused by disseminated pathogens or microbial toxins. Glucocorticoids (GCs) hasten weaning from vasopressors but have adverse effects such as secondary infections and have not consistently improved survival. The anti-inflammatory activity of GCs is mediated through the GC receptor (GR) in a tethered protein-protein interaction with NF- κ B and AP-1 called *trans*-repression. Some related nuclear receptors also demonstrate anti-inflammatory *trans*-repression pathways that could potentially reproduce the benefits of GCs while avoiding their adverse effects, particularly if the anti-inflammatory effect can be directly targeted to the vasculature. The closely related mineralocorticoid receptor (MR) is expressed in endothelium and may possess anti-inflammatory activity in these cells.

The inflammatory response to TNF- α was studied using a human endothelial cell line (EA.Hy926) treated with deoxycorticosterone (DOC), a selective MR agonist, and dexamethasone (DEX), a selective GR agonist.

In silico analysis of a microarray database demonstrated that MR is overexpressed in endothelial cells relative to leukocytes. Both DOC and DEX similarly suppressed TNF- α -induced signaling in an NF- κ B-luciferase reporter system. MR knockdown with siRNA eliminated the anti-inflammatory effect of DOC but not DEX. Real-time PCR experiments showed that DOC suppressed TNF- α -induced transcription of IL-8, ICAM-1, TNF- α , and IL-1 β . Western blotting and electromobility shift assays, respectively, demonstrated that DOC and DEX *trans*-repressed inflammatory signaling without altering NF- κ B nuclear translocation or DNA binding.

These data provide evidence that MR can participate in a non-GR *trans*-repression pathway that blocks inflammation in the endothelium. MR ligands that lack the adverse effect profile of GCs may be useful for treating septic shock.



K. B. BLAINE



K. W. STASER

POSTER A2

Extracellular regulated kinase 1 attenuates stem cell factor signaling in c-Kit-dependent bone marrow cells

KARL WILLIAM STASER, Medical Fellow, Indiana University School of Medicine

Mentor: D. Wade Clapp, M.D., Indiana University School of Medicine

■ Neurofibromin (*Nf1*)-deficient mast cells hyperactively migrate and proliferate in response to stem cell factor (SCF) signaling. Recent data demonstrate that *Nf1*^{+/-} mast cells maintain an inflammatory microenvironment critical to plexiform neurofibroma formation in vivo (Yang, *Cell*, 2008). SCF signals to the mast cell at the c-Kit receptor tyrosine kinase through the Ras-Raf-Mek-Erk pathway, whereby phosphorylated extracellular regulated kinases (Erk1 and Erk2) translocate to the nucleus and promote transcription of pro-growth genes. Few published reports have explored the functional differences between Erk1 and Erk2, and many have assumed their redundancy. Here, we demonstrate an unexpected inhibitory role for Erk1 in SCF intracellular signaling and mast cell function. Bone marrow-derived mast cells (BMMCs) treated with the Erk1/2 inhibitor PD98059 show reduced proliferation, cytokine synthesis, and protein phosphorylation. However, *Erk1*-deficient (*Erk1*^{-/-}) BMMCs demonstrate a hyperactive phenotype similar to the well-published *Nf1*^{+/-} phenotype. *Erk1*^{-/-}, *Nf1*^{+/-} *Nf1*^{+/-}, and *Nf1*^{+/-}; *Erk1*^{-/-} BMMCs treated with SCF proliferate rapidly and hypersecrete the inflammatory cytokines IL-6, TNF- α , CCL-2, CCL-3, and CCL-4, all of which promote pathological inflammation and fibrosis. Biochemically, *Erk1*^{-/-} and *Nf1*^{+/-}; *Erk1*^{-/-} BMMCs demonstrate increased levels of SCF-dependent phospho-Erk2 and phospho- β -catenin. Confocal microscopy reveals heightened translocation of β -catenin from the cortical membrane to the nucleus in the unstimulated and SCF-stimulated mast cell, suggesting novel noncanonical WNT pathway signaling in the mast cell. Moreover, *Erk1*^{-/-}-deficient mice show elevated circulating leukocytes of all lineages in vivo and demonstrate normal to increased hematopoietic colony forming unit potential in vitro. These data suggest an attenuating role for Erk1 in Ras-Mek-Erk signaling and warrant further investigation into SCF-regulated Erk2 function in c-Kit-dependent bone marrow.

POSTER A3

Chlamydia-induced Toll-like receptor 2 signaling leads to increased neutrophil activation and delayed spontaneous apoptosis

LAUREN FRAZER, Medical Fellow, University of Pittsburgh School of Medicine

Mentor: **Toni Darville, M.D.**, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center

■ Toll-like receptors (TLRs) detect microbial infection and participate in the induction of innate and adaptive immune responses. These receptors are found primarily on innate immune cells but are also expressed on many epithelial cells. When intravaginally infected with chlamydia, TLR2 knockout and wild-type mice display similar resolution of infection, but TLR2 knockout mice do not develop oviduct pathology. This indicates that TLR2 activation is essential for the development of oviduct pathology after chlamydial genital tract infection. TLR2 ligands directly stimulate neutrophils, leading to their activation and delayed spontaneous apoptosis. We hypothesize that chlamydia-induced activation of TLR2 induces neutrophil activation and increases neutrophil longevity.

Human neutrophils were infected with a wild-type *Chlamydia trachomatis* strain (D/UW-3/Cx) or a TLR2-signaling-deficient mutant strain (CTD153). After 3 and 6 hours, flow cytometry revealed that neutrophils incubated with D/UW-3/Cx were more activated than those incubated with CTD153 or media with significantly increased CD11 β and CD66b expression at both time points ($P < 0.05$) and significantly decreased CD62L expression at 3 hours. The amount of G-CSF, IL-1 β , IL-8, IL-6, and TNF- α was measured in supernatants at 4 hours and 20 hours, and levels were always higher after infection with D/UW-3/Cx. Release of IL-1 β , IL-6, and TNF- α was significantly ($P < 0.05$) greater at 4 hours, and release of all cytokines tested was significantly elevated at 20 hours. Release of the metalloprotease MMP9 was significantly increased ($P < 0.05$) after 4 hours of incubation with D/UW-3/Cx. Flow cytometric enumeration of apoptosis via TUNEL and microscopic analysis of neutrophil morphology using Giemsa staining revealed incubation with D/UW-3/Cx for 20 hours induced significant protection from spontaneous apoptosis when compared to CTD153 (Giemsa: % apoptotic = $37 \pm 2\%$, D/UW-3/Cx; $78 \pm 2\%$, CTD153; $76 \pm 3\%$, media; $P < 0.01$ for D vs. CTD153 and media).

Chlamydia-induced TLR2 stimulation of neutrophils may promote genital tract pathology by increasing the intensity and duration of proinflammatory molecule production.

POSTER A4

A mouse model of hyper-IgE syndrome (HIES)

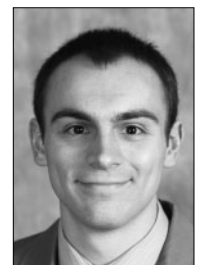
SCOTT STEWARD-THARP, Research Scholar, University of Iowa College of Dentistry, Oxford University

Preceptor: **John J. O'Shea, M.D.**, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health

■ Hyper-IgE syndrome (HIES) is a rare, autosomal-dominant immunodeficiency characterized by eczema, susceptibility to certain infections such as *Staphylococcus aureus* and *Candida*, and severely elevated levels of IgE. Recently, it was shown that heterozygous signal transducer and activator of transcription 3 (STAT3) mutations cause HIES and that patients with these mutations have a deficiency in interleukin 17-secreting CD4⁺ T cells. Despite these significant findings, little is understood about either the cause or effect of the altered immunoglobulin levels or why patients are susceptible to a narrow range of infections only; STAT3 has many diverse functions, and a better understanding of how these functions relate to HIES is necessary. Using recombinering technology, we have generated bacterial artificial chromosome (BAC) transgenic mice that express a STAT3 mutant form isolated from HIES patients. We found that this new mouse model recapitulates several HIES defects related to lymphocyte development, as determined by T cell differentiation and B cell class switching studies. We are currently determining the susceptibility of these mice to infections and their B and T cell responses in vivo.



L. FRAZER



S. STEWARD-THARP

POSTER A5

Role of chemerin, chemokine-like receptor 1, and natural killer cells in particle-mediated joint inflammation

RYAN STEVEN HUSS, Medical Fellow, Stanford University School of Medicine

Mentors: Eugene C. Butcher, M.D., Stanford University School of Medicine, and Brian A. Zabel, Ph.D., Palo Alto Institute for Research and Education

■ The inflammatory response to debris in the joint leads to peri-prosthetic bone breakdown, a problem in up to 15% of patients undergoing total joint replacement. Resident macrophages play a role in peri-prosthetic osteolysis through particle-stimulated cytokine release and differentiation into osteoclasts. Lymphocytes also infiltrate the inflamed joint, and surprisingly, the majority are CD3-CD56+ natural killer (NK) cells. The goal of this project is to identify mechanisms that control NK cell recruitment to the inflamed joint and to characterize functional properties and targets of joint NK cells that may contribute to osteolysis.

We collected joint capsule and synovial fluid (SF) from osteoarthritis patients undergoing primary and revision joint replacement. By flow cytometry, immunohistochemistry, and/or RNA analysis, we found that joint NK cells express high levels of TNF- α receptor CD27. In comparison to blood NK cells, they lack expression of the NK inhibitory receptors NKG2A and CD158a/b, and fail to produce IFN- γ and TNF- α in response to IL-12. However, they do express the NK activating receptors NKp30/44 and NKG2C/D. They also express CXCR3 and CMKLR1, a receptor for the proteolytically activatable chemoattractant chemerin. Joint capsule cultures released pro-chemerin, and SF contained bioactive chemerin, consistent with the presence of chemerin activating proteases in the inflammatory environment.

The detection of bioactive chemerin in inflamed SF, the production of pro-chemerin by joint capsule, CMKLR1 expression by infiltrating NK cells, and previous data showing CMKLR1 expression on blood NK cells together suggest a role for this chemoattractant:receptor pair in osteolysis. Our analysis of joint NK cells shows they are unlikely to contribute to the inflammatory milieu via cytokine secretion, but given their expression of activating receptors, they appear primed for cytolytic killing. Future studies will examine the cytolytic activity of these NK cells and identify their cellular targets, which may include infiltrating T cells or dendritic cells.



R. S. HUSS



R. G. POMERANTZ

POSTER A6

Novel approach to genomic profiling in Sézary syndrome

REBECCA G. POMERANTZ, Medical Fellow, University of Pittsburgh School of Medicine

Mentors: Louis D. Falo, Jr., M.D., Ph.D., and Larisa J. Gaskin, M.D., University of Pittsburgh School of Medicine

■ The objective of this study was to identify specific genetic differences between malignant and nonmalignant CD4+ T cells in a patient with Sézary syndrome (SS). Previous microarray hybridization studies have attempted comparisons of T lymphocytes from SS patients to those of normal controls, yielding widely inconsistent results. Major limitations of this design are 1) significant inherent genetic variability of lymphocyte populations between individuals, which could produce differences in gene expression unrelated to disease state, and 2) heterogeneity of SS patients' circulating T lymphocytes, which do not exclusively consist of the malignant cell population. We addressed these limitations through the use of a novel comparative schema, in which highly purified malignant cells were compared to control T cells from the same patient. Peripheral blood mononuclear cells were obtained by Ficoll-Paque density centrifugation, stained with T cell markers (CD3, CD4, CD45RO) and anti-T cell receptor-V β (TCR-V β) antibodies, and sorted by multiparameter flow cytometry. Malignant cells expressed the dominant TCR-V β , as confirmed by PCR and histopathologic evaluation; control T cells lacked the dominant TCR-V β but were otherwise phenotypically identical (CD3+ CD4+ CD45RO+). These cell populations were compared using the Illumina Sentrix Human-6 expression BeadChip system. Preliminary analysis of microarray data showed changes in the expression of a number of genes known to be associated with carcinogenesis and immunologic dysfunction. The results of this study suggest the feasibility of this novel approach to genomic profiling in SS, in which malignant T cells are compared to control T cells from the same SS patient.

POSTER A7

Effects of CD4 T cell precursor frequency on allospecific memory B cell differentiation

J. BRETT MENDEL, Medical Fellow, Emory University School of Medicine

Mentor: Christian P. Larsen, M.D., Ph.D., Emory University School of Medicine

■ Efforts to improve the survival of engrafted organs have primarily focused on T cell-mediated rejection. Antibody-mediated rejection (AMR) has recently been recognized as an important challenge to improving graft survival. Despite the prevalence of AMR, little is known about the processes leading to the initiation and maintenance of a humoral response against donor-specific antigens. Recent work has shown that CD4+ T cell precursor frequencies play an important role in the development and differentiation program of donor-specific memory CD4+ T cells. Because CD4+ T cells play a role in the development of memory B cell differentiation and maturation, we decided to investigate the effects of CD4+ precursor frequency on donor-specific memory B cells.

Class I MHC H-2k^d restricted CD4+ T cells were harvested from transgenic mice expressing a T cell receptor specific for chicken ovalbumin (DO11 cells). These cells were adoptively transferred into Balb/c mice at high or low precursor frequencies. About twenty-four hours after the adoptive transfer, these mice were given a skin graft from a B6-mOVA (H-2K^b) mouse. Flow cytometry was used to elucidate the effects of CD4 precursor frequency. A fluorescently labeled (H-2K^b) tetramer or ovalbumin was used to identify allospecific B cells.

At high and low precursor frequencies of CD4+ cells, we found that allospecific memory B cells comprised 0.0282% and 0.1238% of the cell population, respectively. These data show that a higher precursor frequency inhibits differentiation and proliferation of linked antigen-specific B cells ($P < 0.05$).

CD4 precursor frequency can have a profound effect on the differentiation and proliferation of allospecific memory B cells. Exploring this relationship as well as other characteristics leading to the memory B cell response will help us gain knowledge that may lead to new therapies for this under-served transplant complication.

POSTER A8

HIV-specific T cell responses for select antigens in exposed, uninfected men who have sex with men

REX G. CHENG, Medical Fellow, Duke University School of Medicine

Mentor: Douglas F. Nixon, M.D., Ph.D., University of California, San Francisco, School of Medicine.

■ The events between initial exposure to HIV-1 and acute infection have not been well characterized. It is unknown whether exposure is linked to immunologic barriers against early stages of infection. While HIV-specific T cell activity is associated with increased viral control in seropositive patients, a possible role in patients who are exposed but remain uninfected is still controversial.

The Pre-Exposure Prophylaxis Initiative cohort (iPrEx) consists of 3,000 seronegative men who have sex with men (MSM). Initial assessment includes a thorough behavioral questionnaire to determine sexual risk. We hypothesize that there are increased HIV-1-specific T cell responses in exposed, uninfected patients compared to unexposed, uninfected controls and that the magnitude is related to route and frequency of exposure.

We studied 32 HIV-1-negative MSM, divided evenly into low- and high-risk exposure groups. Controls are 13 healthy blood donors and 14 HIV+ individuals. Participants are screened for specific CD8+ T cell responses against Gag clade B, Gag clade C, Nef, Vif, and Pol genes (integrase, reverse transcriptase, protease) by IFN- γ ELISPOTs.

Of the 32 participants, none responded to Gag B, Gag C, or Vif. One participant made a striking response against Nef. Three responses were made against integrase. Two of those three made responses to RT, and all three made strong responses to protease. The mean protease response was significantly greater than both controls, suggesting that the exposed, uninfected population recognizes a different set of HIV-1 epitopes not seen in seropositive individuals. These responses may possibly be protective against HIV infection.



J. B. MENDEL



R. G. CHENG

POSTER A9

Loss of HIV-specific memory B cell response following initiation of antiretroviral therapy

JENNY CHEN, Research Scholar, Indiana University School of Medicine

Preceptors: Anthony S. Fauci, M.D., and Susan Moir, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ We recently described HIV-associated B cell exhaustion in HIV-viremic individuals that was accounted for by a distinct subset of tissue-like memory B cells. This subset bore signature features of exhaustion, including increased expression of multiple inhibitory receptors, a stunted replication history and immunoglobulin diversity, and poor proliferative capacity. Given that the HIV-specific memory B cell response was enriched within tissue-like memory B cells, we suggested that B cell exhaustion in HIV-viremic individuals contributes to the inefficiency of their HIV-specific antibody response. In the present study, we performed longitudinal analyses of B cell subsets following the initiation of ART in HIV-infected individuals.

Tissue-like memory B cells manifesting features of exhaustion were observed in the peripheral blood of HIV-infected individuals, representing on average 20% of total mature B cells compared to <5% in uninfected individuals. Following initiation of ART, these percentages decreased within 6 months to <10%, consistent with previous observations that ongoing viral replication is responsible for the induction of exhausted B cells. Furthermore, the frequency of HIV-specific ASCs decreased to undetectable levels both as a result of the decrease in percentage of tissue-like memory B cells (which contained the bulk of the HIV-specific ASCs) and a decrease in HIV-specific ASCs within the classic-memory B cell compartment. In contrast, the frequencies of nonspecific and recall antigen-specific ASCs were either stable or increased following ART in this latter compartment.

Whereas ART leads to a normalization of B cell subsets in HIV-infected individuals, it does not lead to a redirection of the HIV-specific response into the classic memory B cell compartment as would be required for an effective protective response.



J. CHEN



B. JAGGER

POSTER A10

Influenza pandemic evolution: the role of the viral polymerase protein PB1

BRETT JAGGER, Research Scholar, Indiana University School of Medicine

Preceptor: Jeffery K. Taubenberger, M.D., Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Influenza A virus (IAV) unpredictably causes worldwide pandemics in humans, resulting in considerable morbidity and mortality. Prior pandemic viruses have resulted from reassortment events between avian IAVs and human IAVs, a process that may yield progeny that are antigenically novel and therefore able to spread readily in the human host. However, genetic analyses of the last three pandemic viruses have also shown that in addition to the surface antigens hemagglutinin and neuraminidase, the viral polymerase basic protein-1 (PB1) has likewise been avian-derived. Further, in contrast to the evolutionary stasis observed in avian hosts, IAV PB1s in human circulation are subjected to positive selection pressure, accumulating coding mutations that deviate from the avian consensus. Given PB1's central role in IAV polymerase function, these observations suggest that PB1 contributes to pandemic viral fitness.

To address this question, we assayed the *in vitro* function of representative avian, interpandemic, and pandemic PB1s isolated from 1940 to 1968 by using luciferase and GFP reporters as surrogates of polymerase activity. It was hypothesized that the interpandemic human PB1s would demonstrate significantly lower activity than both avian and pandemic PB1s. Indeed, when normalized to a homologous interpandemic polymerase complex, avian PB1s increased activity by 3- to 4-fold, while the pandemic virus-derived PB1s produced increases of 2-fold for the 1957 pandemic PB1 and 3.5-fold for the 1968 pandemic PB1. These differences were statistically significant.

Avian- and pandemic-derived PB1s increased the expression of a reporter protein over interpandemic-derived PB1s in an *in vitro* model of influenza polymerase function. This observation supports the hypothesis of a role for PB1 in determining the fitness of reassortant pandemic viruses. Application of reverse genetics techniques to determine the relevance of these observations to live virus phenotypes is ongoing.

POSTER A11

Investigation of cellular transactivator stimulatory protein-1 on the varicella-zoster virus open reading frame 63 promoter region

MAKEDA L. ROBINSON, Medical Fellow, Stanford University School of Medicine

Mentor: Ann M. Arvin, M.D., Stanford University School of Medicine

■ Varicella-zoster virus (VZV) is a human alpha-herpesvirus that causes varicella during primary infection, persists in sensory ganglia, and may reactivate from latency to cause zoster. The transcript for the open reading frame 63 gene is the most abundantly expressed in the sensory ganglia during the latent phase of the virus life cycle. The ORF63 is located between nucleotides 110581 and 111417 in the internal repeat region. It is duplicated in the terminal repeat region as ORF70 (nucleotides 118480–119316). When these ORFs are deleted from the genome, VZV is impaired for replication in both melanoma cells and fibroblasts. A binding site for the transactivating cellular protein, stimulatory protein-1 (sp-1), is encoded in the promoter region of ORF63 and ORF70.

We evaluated the role of ORF63 and ORF70 in VZV replication, using recombinant VZV cosmids and PCR-based mutagenesis to make point mutations of the binding site for sp-1 in the promoter region of these ORFs. VZV was recovered when cosmids with these mutations were transfected into melanoma cells along with the three intact VZV cosmids. These mutations yielded viral plaques. The recovered virus retained infectivity in human embryonic lung fibroblasts (HELFL) cells. The mutated virus was then evaluated by confocal immunofluorescence for the spatial expression of IE62, IE63, and gE in infected melanoma cells at 48 hours postinfection. The results to date indicate that point mutations of the sp-1 transactivating site in ORF63 and ORF70 slow but do not inhibit productive replication; however, the localization of the sp-1 mutant is similar to the wild-type virus. Evaluation of the mutant in skin xenografts in SCID mice is in progress.

POSTER A12

Magnetic resonance imaging in the detection and therapeutic monitoring of hematogenous *Candida* meningoencephalitis

JESSICA M. VALDEZ, Research Scholar, University of New Mexico School of Medicine

Preceptor: Thomas J. Walsh, M.D., National Cancer Institute, National Institutes of Health

■ Hematogenous *Candida* meningoencephalitis (HCME) is a life-threatening complication of immunocompromised children, especially those receiving chemotherapy and those in neonatal intensive care units. The infection is associated with severe morbidity and mortality. Complications include seizure, abscesses, ventricular hemorrhage, delayed developmental milestones, and impaired cognition, and death can occur. Treatment of this form of invasive candidiasis in children with cancer or other immune impairments is difficult and fraught with multiple recurrences. Detection of infection is also difficult, as cerebrospinal fluid (CSF) and cultures are insensitive and conventional diagnostic imaging lacks adequate resolution. A pressing need exists for advances in diagnostic imaging modalities to improve diagnosis and therapeutic monitoring of HCME. Using a well-characterized rabbit model of HCME we are developing a sensitive MRI protocol to aid in the early detection and therapeutic monitoring of the lesions of HCME. We are investigating the role of the immune response and characterization of the lesions. Using the in vivo rabbit model in parallel with in vitro MRI studies, we will elucidate the relative contributions of the cellular components of the inflammatory response to the MRI signal, including monocytes, endothelium, astrocytes, and *Candida* cells. Infection of the nonneutropenic rabbit model is established with *Candida albicans* NIH isolate 8621. The MRI characteristics of nonneutropenic anesthetized hosts include T1 precontrast; T1 postcontrast, utilizing gadolinium and superparamagnetic iron oxide particles (SPIO); T2 for fluid accumulation; magnetization transfer for macromolecule concentration; and spectroscopy for acquiring metabolic information from the rabbit CSF. Antifungal therapies, which are initiated 48 hours after inoculation, include deoxycholate amphotericin B (DAMB; 0.5 mg/kg and 1 mg/kg) and fluconazole (10 mg/kg). The combined results of these studies will provide a scientific foundation for the development of a clinical protocol designed to detect early lesions of HCME and provide a basis for therapeutic intervention and monitoring in immunocompromised children.



M. L. ROBINSON



J. M. VALDEZ

POSTER A13

Effect of oxathiazolones and their derivatives on survival of *Plasmodium falciparum*

SHAKA J.D. BAHADU, Medical Fellow, Weill Cornell Medical College

Mentor: Carl Nathan M.D., Weill Cornell Medical College

■ Malaria is a life-threatening disease caused by the protozoan parasite genus *Plasmodium*. According to the World Health Organization, in 2006 alone, there were approximately 250 million reported cases. Close to 900,000 people died, mostly children. The specter of drug resistance remains present, and currently there are no viable alternative therapies to artemisinin-derived compounds. We have synthesized and tested a series of proteasome inhibitors called oxathiazolones against *Plasmodium falciparum* by measuring parasite viability. We utilized a transgenic line of parasites that expresses firefly luciferase, providing a rapid, convenient, and accurate assay for parasite survival in the presence of candidate compounds. *P. falciparum* cultures (200 μ L) were used to screen compounds in 96-well plates. Compounds were assayed in duplicate and later triplicate at concentrations ranging from

25 to 50 μ M. Plates were incubated for 48 hours in a vacuum-sealed container with a modified atmospheric condition (CO₂ 5%, O₂ 5%, N₂ 90%) designed to optimize parasitic growth. At 48 hours, samples were lysed, a luciferin reagent was added, and luminescence signal was measured. Using this system, we identified putative proteasome inhibitors (and one derivative that is not a proteasome inhibitor) that substantially decreased survival of *P. falciparum* in culture. Thirty-eight compounds were screened. Seven compounds—HT1059, HT1074, HT1213, HT1231, HT1232, HT2050, and HT2082—demonstrated >50% decrease in parasite survival compared to untreated samples and controls (DMSO). Two compounds—HT1059 and HT1074—showed virtually complete inhibition of parasite viability (HT1059 at 50 μ M, 0.049 relative light units [RLU]; HT1074 at 50 μ M, 0.29 RLU vs. DMSO control, 43.4 RLU). Oxathiazolones were designed to be mycobacteria-specific proteasome inhibitors. Serendipitously, some of these compounds were effective against *Plasmodium* survival. Future work will determine the mechanism of action of these compounds within the parasites, in hopes of leading to novel chemotherapies for malaria.



S. J. D. BAHADU

POSTER B1

Survival of the perivascular niche following irradiation reveals heterogeneity of the radiation response in glioblastoma multiforme in vivo

KARIM Y. HELMY, Medical Fellow, University of Medicine and Dentistry of New Jersey–New Jersey Medical School

Mentor: Eric C. Holland, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center

■ Glioblastoma multiforme (GBM) is the most common and most aggressive primary brain tumor in adults, with a median survival of only 12–15 months. Current standard-of-care for GBM patients includes surgical resection followed by radiation therapy combined with temozolomide chemotherapy. Despite aggressive treatment, most GBM patients suffer recurrence or progression. In this study, we sought to evaluate the heterogeneity of the radiation response among different cell types within the tumor using a mouse genetic model of GBM driven by platelet-derived growth factor (PDGF). We then characterized the transcriptional and translational response to radiation in subsets of cells within the tumor by microarray profiling of total RNA from sorted cells and polysome-associated RNAs from these same cell populations using translating ribosome affinity purification (TRAP) technology.

Mice harboring GBM tumors were exposed to 10 gray (Gy) whole-body irradiation and sacrificed at various time points following treatment for analysis of the radiation response. Twenty-four hours after irradiation, olig2-positive cells that comprise the bulk of the tumor had significantly higher levels of apoptosis than nestin-positive cells residing in the perivascular niche (PVN). Over the course of six days following radiation treatment, the percentage of olig2-positive cells in the tumor decreased significantly, and there was a concomitant expansion of the nestin-positive PVN. Microarray analysis of olig2-positive cells before and after radiation revealed that the pro-apoptotic and cell cycle arrest phenotype observed histologically is regulated differentially at the transcriptional and translational levels.

Ongoing studies seek to evaluate the source and transformation status of the expanding PVN following irradiation and assess the contribution of these surviving cells to tumor repopulation following radiotherapy. Understanding the mechanisms of the radiation response of the different cell populations that comprise GBM tumors may facilitate the development of agents that radiosensitize these cells and lead to improved outcomes for GBM patients.

POSTER B2

Radiosensitization of glioblastoma multiforme by modulation of Met signaling with human anti-hepatocyte growth factor monoclonal antibody

IAN M. BUCHANAN, Research Scholar, The Warren Alpert Medical School of Brown University

Preceptor: Kevin Camphausen, M.D., National Cancer Institute, National Institutes of Health

■ Of the ≈ 1.5 million cancers diagnosed in the United States annually, 75% receive radiation therapy (RT) in the course of treatment. Normal tissue toxicity is the limiting factor for RT. Taking advantage of biology unique to tumors, pharmaceutical radiation sensitizers increase radiation's impact on neoplastic tissue specifically, while leaving normal tissue unaffected. The hepatocyte growth factor (HGF)/Met signaling pathway is upregulated in many cancers, with significant effects, including promitogenic, prometastatic, and anti-apoptotic signals. It is our hypothesis that modulation of the Met pathway will specifically increase the cytotoxic effects of RT in cancer cells.

As RT is still the firstline therapy in glioblastoma multiforme, the U87-MG cell line was the model system selected; 24 h of pretreatment with a recombinant human anti-HGF monoclonal antibody prior to 2 Gy of ionizing radiation was the standard treatment protocol. Drug treatment was shown, by Western blot, to prevent activation of Met at 1, 6, and 24 h following radiation. Clonogenic survival assays showed a dose-enhancement factor of 1.2 with no toxicity from drug alone. Quantitation of double-strand breaks by the phospho-histone marker γ -H2AX indicated a statistically significant increase at 6 and 24 h postradiation in drug-treated cells (10.4 vs. 7.4%, $P < 0.001$; 5.8 vs. 3.8%; $P < 0.001$). A nominal increase in apoptosis was noted at 72 h after radiation; however, at both 48 and 72 h, a statistically significant increase of cells undergoing mitotic catastrophe was appreciated (10.2 vs. 6.8%, $P = 0.02$; 14.1 vs. 8.8%, $P = 0.01$). Pretreatment with drug caused no cell cycle redistribution, nor was the G2-checkpoint affected following radiation.

These results indicate that modulation of Met signaling with this agent holds promise as a radiation-sensitizing strategy. Our data indicate that DNA repair processes downstream of the Met signaling cascade are impaired, leading to increased cell death through mitotic catastrophe.

MONDAY
ATRIUM



K.Y. HELMY



I. M. BUCHANAN

POSTER B3

Relevance of Polycomb group-mediated epigenetic modifications in glioblastoma multiforme pathophysiology

CHIBA ENE, Research Scholar, Indiana University School of Medicine

Preceptor: Howard A. Fine, M.D., National Cancer Institute, National Institutes of Health

■ Epigenetic modifications such as DNA methylation have been implicated in mediating tumorigenicity in numerous cancer types. Other alterations such as posttranslational modifications to histones have also been linked to cancer. Histones are no longer considered to be simple DNA-packaging proteins; they are now recognized as being dynamic regulators of gene activity that undergo many posttranslational chemical modifications, including acetylation, sumoylation, phosphorylation, ubiquitylation, and methylation. The trithorax and Polycomb group (PcG) proteins are chromatin modifiers that play an essential role in mediating heritable epigenetic repression of gene activity. PcG proteins form three different classes of complexes, including the Polycomb repressive complex 2 (PRC2). Tri- and dimethylation of histone 3 lysine 27 (H3K27) is believed to be the key mechanism by which PRC2 regulates cellular processes such as development, differentiation, and proliferation. In prostate cancer, PRC2-mediated H3K27 trimethylation has been shown to be predictive of clinical outcomes independent of tumor stage, PSA levels, and capsule invasion. However, the functional relevance of this modification in tumor pathophysiology remains elusive.

Glioblastoma multiforme (GBM) is the most common and most aggressive type of brain tumor in adults. Despite aggressive chemotherapy and radiation, median survival upon diagnosis is 14 months and the 5-year survival rate is 10%. Unfortunately, over the past 20 years, little or no improvement in the survival of patients with this condition has been seen; therefore, the development of more effective therapies is desperately needed. With the discovery of cells within cancers such as GBM with stem cell-like properties, termed tumor-initiating cells (TICs), there is a paradigm shift in the way we study these tumors. From a therapeutic standpoint, several studies have shown that a stem cell-like phenotype confers resistance to conventional therapy, making these cells a great model for studying disease pathophysiology and possibly a therapeutic target. Our study investigates the functional relevance of PRC2-mediated epigenetic modifications in glioblastoma TICs.



C. ENE



R. L. CHARD

POSTER B4

Vascular endothelial growth factor C (VEGF-C) is important in the development and metastasis of head and neck squamous cell carcinoma

RACHEL L. CHARD, Research Scholar, Oregon Health and Science University School of Medicine

Preceptor: J. Silvio Gutkind, Ph.D., National Institute of Dental and Craniofacial Research, National Institutes of Health

■ Head and neck squamous cell carcinomas (HNSCCs) are the sixth most common cancers worldwide. Though advances in prevention and treatment have increased survival rates in other cancers, the 5-year survival rate for HNSCC patients, approximately 50%, has remained virtually unchanged for more than 30 years. In search of molecules with potential therapeutic benefit, we have conducted a comprehensive genomic and proteomic analysis of laser capture microdissected cancer and stromal cells from a large collection of HNSCC frozen tumors from patients of distinct metastatic status. We observed that the expression of vascular endothelial growth factor C (VEGF-C) is a prominent feature in the most metastatic HNSCC lesions. VEGF-C, a member of VEGF family of growth factors, plays a key role in the process of lymphangiogenesis or the growth and proliferation of the lymphatic vasculature. This prompted us to focus on the dysregulation of lymphangiogenesis pathways in HNSCC. We have recently established rapid mouse orthotopic models of HNSCC invasion in the tongue and the intra-vital imaging approaches to monitor the process in live animals, thereby enabling us to address the role of VEGF-C in HNSCC metastasis. Using a lentiviral delivery system, we expressed two unique sequences of shRNA against VEGF-C to knock down its release in OSCC3, a well-characterized HNSCC cell line of high metastatic potential. We then injected these cells into SCID/Nod mice using our validated model of HNSCC metastasis, observing differences in tumor volume, metastasis, and vasculature density. In parallel, we characterized the effects of VEGF-C secreted by OSCC3 control and VEGF-C knockdown cells on the ability of immortalized lymphatic endothelial cells to proliferate and migrate. We aim to demonstrate that the release of VEGF-C by tumor cells is important for the process of lymphatic metastasis, and, consequently, that VEGF-C signaling should be further investigated as a therapeutic target in HNSCC.

POSTER B5

TGF- β receptor III is downregulated by Δ Np63 and methylation and contributes to altered TGF- β and NF- κ B signaling and the malignant phenotype in head and neck squamous cell cancer

FREDERICK WANG, Research Scholar, Yale School of Medicine

Preceptor: Carter Van Waes, M.D., Ph.D., National Institute on Deafness and Other Communication Disorders, National Institutes of Health

■ Alterations in the function of the TGF- β signaling pathway have been implicated in the development and progression of many cancers, including head and neck squamous cell carcinoma (HNSCC). Microarray analysis of a panel of HNSCC cell lines revealed a distinct pattern of TGF- β receptor expression that segregated based on p53 mutation status. HNSCC cell lines deficient in wild-type p53 expression had decreased while those with mutant p53 expression had increased TGF- β receptor III (T β R3) mRNA transcripts and protein relative to human epidermal keratinocytes (HEKA), leading us to investigate the role of T β R3 in tumorigenesis. No mutations affecting amino acid sequence were observed. The cellular response to TGF- β 1 and TGF- β 2 was attenuated in HNSCC cell lines as assayed by cell proliferation, suggesting that the altered expression of T β R3, which has a high affinity to TGF- β 2, may contribute to the malignant phenotype. TGF- β 1 and TGF- β 2 also differentially modulated the classical downstream genes of TGF- β pathway, such as PAI-1, MMP2, and MYC. Δ Np63, a TP53 family member with oncogenic activity, suppressed T β R3 gene expression and the downstream gene SMAD7. Treatment with 5-azacytidine also increased T β R3 mRNA levels, suggesting that methylation may play a role in decreased T β R3 levels. Overexpression of T β R3 altered NF- κ B and PAI-1 reporter activity, demonstrating that T β R3 is involved in signaling through both NF- κ B and TGF- β pathways. The expression levels of several downstream targets of both signaling pathways were also affected, including ZEB2, a transcriptional repressor of E-cadherin important in regulating epithelial-mesenchymal transition (EMT). In summary, this is the first study to our knowledge to demonstrate that altered T β R3 expression in subsets of HNSCC may contribute to aberrations in the signaling of both TGF- β and NF- κ B pathways, leading to loss of negative control and aggressive malignancy.

POSTER B6

Genetic analyses of constitutive NF- κ B activation in diffuse large B cell lymphoma

PAUL B. ROMESSER, Research Scholar, Boston University School of Medicine

Louis Staudt, M.D., Ph.D., National Cancer Institute, National Institutes of Health

■ Diffuse large B cell lymphoma (DLBCL), the most common variant of non-Hodgkin's lymphoma, is a highly aggressive lymphoma. One-third of DLBCL cases are classified molecularly as activated B cell-like (ABC DLBCL), which carries a particular dismal prognosis. ABC DLBCL is characterized by constitutive NF- κ B activation, a regulator of B cell activation. Inducible RNA interference screens in ABC DLBCL cell lines identified CARD11 as a key upstream NF- κ B signaling protein necessary for survival. Mutational variants of *CARD11* identified in DLBCL patient samples were shown functionally to activate the NF- κ B pathway. These *CARD11* mutations clustered within the coiled-coiled domain, an evolutionary conserved domain that mediates CARD11 oligomerization and activation. Much is unknown regarding the regulation of CARD11 and the role it plays in modulating NF- κ B. Although it has been shown that the coiled-coiled domain has regulatory roles, detailed molecular mechanisms are missing. Here, we show a high-throughput genetic screen for the systematic identification of novel *CARD11* mutations. This approach has provided additional *CARD11* candidate mutations for further functional analyses. Future biochemical analyses of these mutations are important not only for the understanding of CARD11's role in the pathogenesis of ABC DLBCL, but also for understanding CARD11's natural role in the regulation of B cell activation.



F. WANG



P. B. ROMESSER

MONDAY
ATRIUM

POSTER B7

Induction of functionally competent interleukin-21 receptor in B cells from chronic lymphocytic leukemia patients

REBEKAH BROWNING, Medical Fellow, The Ohio State University College of Medicine

Mentor: John C. Byrd, M.D., The Ohio State University

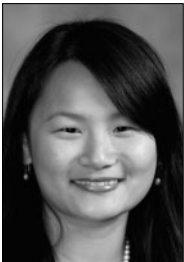
■ Interleukin 21 (IL-21), a member of the common gamma chain family of cytokines, mediates receptor expression-dependent apoptosis in B cells from chronic lymphocytic leukemia (CLL) patients. We hypothesize that upregulation of functionally competent IL-21 receptor expression using clinically relevant agents will improve response to IL-21. We describe here evidence for induction and functional relevance of IL-21R using a novel CpG oligodeoxynucleotide (CpG-ODN) that we are developing for clinical use in CLL.

Immunoblot analysis using anti-IL-21R- α antibody showed a time-dependent induction of IL-21R in CD19+ primary CLL B cells treated with CpG-ODN at as early as six hours. Quantitative real-time PCR analysis revealed that this upregulation occurs at the transcriptional level. IL-21R transcript levels showed a mean 16-fold change over media control at three hours of CpG-ODN treatment ($n=5$) and a 6-fold change at 24 hours ($n=7$). In order to establish the signaling competence of CpG-ODN-induced IL-21R, we treated CLL cells with CpG-ODN followed by IL-21 and evaluated phosphorylation status of known downstream targets of IL-21R signaling, including STAT1, STAT3, and JAK1, by immunoblot analysis. Increased IL-21-induced phosphorylation of these proteins was observed in the CpG-ODN pretreated samples compared to untreated controls. The functional competence of CpG-ODN-induced IL-21R to mediate IL-21-induced apoptosis was evaluated by Annexin V-FITC/PI staining. For a subset of patient cells that showed little or no response to either CpG or IL-21 alone, concentrations of CpG-ODN that induced upregulation of the IL-21R resulted in IL-21-mediated cytotoxicity.

These studies provide evidence for potential clinical application for the CpG-ODN or other IL-21R inducing agents for combination therapy with IL-21. Ongoing studies are aimed at characterizing the endogenous and induced regulation of IL-21R promoter in CLL B cells using biochemical and molecular approaches.



R. BROWNING



A. CAI

POSTER B8

Mutated BCR-ABL as immunologic targets in chronic myelogenous leukemia (CML)

ANN CAI, Medical Fellow, Harvard-MIT Division of Health Sciences and Technology

Mentors: Catherine J. Wu, M.D., and Jerome Ritz, M.D., Harvard Medical School

■ Although imatinib successfully induces remission in chronic myelogenous leukemia (CML), >95% of patients demonstrate persistent molecularly detectable disease with a 2–3% yearly relapse rate. Imatinib inhibits CML-specific BCR-ABL kinase activity, but BCR-ABL mutations impeding drug binding effect imatinib resistance. Historically, CML has proven highly immunoresponsive; 75–80% of relapsed patients achieve durable molecular remission following infusion of donor lymphocytes. Specific and nontoxic immunotherapy is one promising approach to eradicating persistent disease. Because imatinib-resistant patients typically harbor mutated BCR-ABL and wild-type BCR-ABL-derived peptides do not consistently elicit immunoresponses, we seek to understand whether mutated BCR-ABL-derived peptides could serve as immunogenic tumor-specific targets.

Using conventional sequencing or polony assay (in situ solid-phase PCR amplifications of individual DNA molecules), we identified the three most common BCR-ABL mutations (T315I, M351T, E255K) in nine patients with increased *BCR-ABL* levels following imatinib treatment. By tiling 9mers around each mutation and using an IEDB server for HLA-A and HLA-B haplotypes, we predicted peptide-MHCI allele binding for each patient. We identified 16 peptides with high predicted binding ($IC_{50} < 10,000$ nM); two E255K-derived peptides had the best binding scores and were predicted to bind HLA-A3 better than their parental peptides. Using a competitive MHC peptide binding assay, we confirmed 10-fold greater binding affinity of mutated relative to parental peptide. Three of nine patients were HLA-A3+ and had the E255K mutation. HLA-A3+ tetramers for these peptides have been generated (NIH Tetramer Core Facility, Emory).

Ongoing studies focus on generating peptide-specific T cell lines for testing dendritic cell processing and presentation of these epitopes and on detecting peptide-specific T cells by tetramer staining in relapsed CML patients following subsequent allotransplantation. If patient T cell responses to mutated BCR-ABL-derived epitopes are detected, then this would support the development of personalized vaccination strategies for the treatment of drug-resistant CML.

POSTER B9

Low-fat diet reduces the progression of established bone metastases in mice

TIMOTHY VAN JOHNSON, Medical Fellow, Emory University School of Medicine

Mentors: Leland W.K. Chung, Ph.D., Winship Cancer Institute, and Viraj A. Master, M.D., Ph.D., Emory University School of Medicine

■ With an incidence of ~220,000 cases/year and a lifetime risk of ~20%, adenocarcinoma of the prostate (CaP) is the number 1 noncutaneous malignancy and the number 2 cause of cancer-related death in American men. Almost one-third of CaP patients develop metastases (mets), most commonly to bone, which doubles both the risk of overall fractures and the cost of treatment. Currently, median survival with bone mets is 21–33 months. Compelling clinical data from recent cohort studies suggest that a low-fat diet may reduce mortality in metastatic adenocarcinoma of the breast and colon, but no such data exist for CaP bone mets. Based on previous protocols for establishing bone mets, we injected 60 athymic NCr-nu/nu male mice with $0.5\text{--}2 \times 10^6$ ARCaPE cells intratibially. Mice were randomized to receive either a low-fat (LF: 10% fat) or Western diet (WD: 40% fat). Mice were fed a modified paired feeding protocol, and weights and caloric intake were measured biweekly to ensure equal caloric intake across all groups. Thirty mice (15 from each group) were sacrificed after 12 weeks, to determine actual tumor size at the same time point. Remaining mice were sacrificed once their tumors reached 1,100 mm³. LF and HF mice consumed an equal number of calories throughout the experiment and consequently maintained statistically identical body weights. Twelve weeks after tumor injection, bone mets in LF mice were 33% smaller than in WD mice ($P < 0.05$). Additionally, LF mice on average had a 20% longer survival than WD mice ($P < 0.05$). These data suggest that a low-fat diet may reduce the progression of established bone mets, a novel finding that may provide hope and opportunity to patients afflicted with this disease.

POSTER B10

MicroRNA expression profiles associated with cancer-specific mortality in colon adenocarcinoma

JASON E. HAWKES, Research Scholar, University of Utah School of Medicine

Preceptor: Curtis C. Harris, M.D., National Cancer Institute, National Institutes of Health

■ Colon cancer is the third most common cancer, accounting for approximately 10% of all cancer-related deaths. The discovery of novel colon cancer biomarkers and therapeutic targets is essential to the management of this disease. MicroRNAs represent a group of molecules associated with tumorigenesis and have potential as diagnostic biomarkers and therapeutic targets. We recently reported that individual microRNAs are differentially expressed in colon adenocarcinomas and associated with poor survival (Schetter et al., *JAMA* 2008). In that study, microRNA microarray expression profiling of tumor and paired nontumorous tissues was performed on a Maryland cohort of 84 patients with colon adenocarcinoma. Thirty-seven microRNAs were differentially expressed in colon tumors from this test cohort ($P < 0.001$). Further analysis determined that miR-20a, miR-21, miR-106a, miR-181b, and miR-203 were also associated with poor survival in the test cohort. Whereas these 5 microRNAs were also found to be overexpressed in a second, independent Hong Kong cohort of 113 patients ($P < 0.001$), only miR-21 expression was associated with prognosis ($P = 0.001$). Overexpression of miR-21 was also associated with poor therapeutic outcome in stage II and III colon cancer ($P = 0.003$). We are currently examining these microRNAs in a new, independent Maryland cohort of 95 colon adenocarcinoma patients from the greater Baltimore area, using updated microRNA microarrays. We are testing the hypothesis that microRNAs are differentially expressed in colon tumors and that microRNA expression patterns are associated with cancer survival. We are attempting to validate the 37 previously identified microRNAs differentially expressed in tumors and the 5 microRNAs associated with poor survival in our original Maryland cohort. The updated microRNA microarrays also allow us to examine an additional 130 previously unstudied microRNAs and any new associations between these microRNAs and colon tumors. To our knowledge, this is one of the largest studies to date analyzing microRNA profiles in colon cancer tissues.



T.V. JOHNSON



J. E. HAWKES

MONDAY
ATRIUM

POSTER B11

Development of mouse tumor models for multidrug resistance

MICHELLE SAMUEL, Research Scholar, University of Pennsylvania School of Veterinary Medicine

Preceptor: Michael Gottesman, M.D., National Cancer Institute, National Institute of Health

■ The development of multidrug resistance (MDR) to a variety of anticancer drugs is a major obstacle for cancer drug efficacy. P-glycoprotein (P-gp, MDR1), an ATP-dependent efflux pump is responsible for reduced intracellular accumulation and reduced cytotoxicity for many anticancer drugs and is often implicated in clinical chemotherapy resistance. Preclinical drug evaluation of true efficacy against MDR tumors is optimally assessed in mouse xenograft models. Such models have been used for many years to assess the effects of multidrug resistance on cancer drug efficacy. We have observed that MDR tumor cell lines that express significant levels of P-gp in vitro typically lose P-gp expression once established as xenografts. This has important implications for a large number of both completed and ongoing xenograft studies. In order to characterize P-gp loss and establish a human cell-derived MDR mouse xenograft model, MDR cell lines KB 3-1, KB 8-5, KB 8-5-11, HCT-15, MCF7/ADR, and NCI/ADR-RES were implanted subcutaneously in nude athymic and SCID mice. Tumor tissue was evaluated for P-gp expression to assess host influence on P-gp expression. Additional xenografts (using the same MDR cell lines) will be established and dosed regularly with low concentrations of doxorubicin regularly administered to determine whether P-gp expression can be induced in an analogous fashion to cell culture and clinical MDR strategies and to serve as a useful MDR mouse model.



M. SAMUEL



P.W. BLAKE

POSTER B12

Clinical and molecular genetics investigations of Brooke-Spiegler syndrome and molecular modeling of CYLD mutations

PATRICK W. BLAKE, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Preceptor: Jorge R. Toro, M.D., National Cancer Institute, National Institutes of Health

■ Brooke-Spiegler syndrome (BSS) is a rare autosomal dominant predisposition to cutaneous appendageal neoplasms associated with germline mutations in the cylindromatosis (*CYLD*) gene. We conducted a comprehensive evaluation of BSS and the molecular effects of *CYLD* mutations using a multidisciplinary clinical, genetic, and molecular approach. We have examined and screened BSS families for internal malignancy, because to our knowledge, such an association has not been previously reported. We identified several families in whom BSS and colorectal cancer cosegregated over two generations, suggesting that these two conditions may be associated. Molecular studies appear to support this association. In addition, our meta-analysis of the literature revealed that the six missense *CYLD* mutations reported to date occur within the ubiquitin-specific protease (USP) domain of *CYLD* and 5/6 mutations were associated with multiple familial trichoepithelioma. Using modeling software, we inspected the structure of the *CYLD* USP domain to identify possible structural effects of *CYLD* missense mutations. Our analyses revealed that four of the mutated residues (G596, D681, P904, and D941) are arranged in a ring around the catalytic site. The G596D, D681G, and D941V mutations involve the addition or removal of an ionizable side chain. P904L may affect the mobility of the loop in which it resides, and E747G might disrupt protein-protein interactions. To evaluate the functional consequences of these mutations, we plan to transfect mutant constructs into fibroblasts and HEK-293 cells to assess deubiquitination of *CYLD* targets and downstream NF- κ B activity. Our investigations revealed that BSS appears to be associated with colorectal cancer and exhibits a genotype-phenotype correlation. Missense mutations in the USP domain of *CYLD* have predicted functional consequences associated with multiple trichoepitheliomas. Determining that BSS is associated with an increased risk of internal malignancy will help us to identify at-risk patients and improve our understanding of cutaneous and internal organ tumorigenesis.

POSTER B13

Induction of a mesenchymal phenotype in tumor cells and a cytokine signature associated with tumor progression

MARIANNE D. CASTILLO, Research Scholar, University of Medicine and Dentistry of New Jersey–New Jersey Medical School

Preceptors: Jeffrey Schlom, Ph.D., and Claudia Palena, Ph.D., National Cancer Institute, National Institutes of Health

■ Understanding the molecular mechanisms of metastasis is a major step in designing effective cancer therapeutics. Our laboratory has previously demonstrated that the T-box transcription factor Brachyury is highly expressed in various human tumors and tumor cell lines of epithelial origin and not in most normal human adult tissues. During embryogenesis, Brachyury is necessary for normal mesoderm formation, a typical example of an epithelial-to-mesenchymal transition (EMT). By performing Brachyury overexpression and siRNA inhibition experiments with various human tumor cell lines, we have been able to demonstrate that Brachyury imparts to tumor cells the characteristics of mesenchymal cells, including migratory and invasive properties. In this study, we further investigated the mechanisms involved in Brachyury-mediated tumor progression and the impact of tumor EMT on the microenvironment and immune responses to the tumor. By using a pancreatic (PANC-1) and a breast (MCF-7) carcinoma cell line, we demonstrated that Brachyury overexpression induces a cytokine signature associated with cancer cell migration, invasion, and angiogenesis. Among the cytokines highly secreted by Brachyury-positive versus Brachyury-negative tumor cells were IL-6, IL-8, GRO, RANTES, all implicated in tumor cell migration and invasion; the bone-remodeling molecule osteoprotegerin; and the pro-angiogenic factors angiogenin, VEGF, and PlGF. Our results also demonstrated that Brachyury expression in tumor cells regulates the cell surface expression of various immune-associated molecules, which are critical for the establishment of an effective antitumor immune response, such as ICAM-1, LFA-3, and MHC-class I. In summary, we provide insight into possible mechanisms by which tumors undergoing Brachyury-mediated EMT may potentially modulate their microenvironment.

POSTER B14

Exploring mechanisms in epigenetic regulation of epidermal homeostasis

JENNIFER K. CHEN, Medical Fellow, Johns Hopkins University School of Medicine

Mentor: Paul A. Khavari, M.D., Ph.D., Stanford University School of Medicine

■ Epidermal homeostasis is disrupted in many human diseases, including psoriasis, cancer, and chronic wounds. Understanding the control of epidermal growth and differentiation at the level of gene regulation is of vital importance both to understanding disease pathogenesis and as a rational foundation for the development of new treatment strategies. Regulators of epidermal homeostasis include well-known players such as p63, a member of the p53 family of proteins known to promote proliferation and differentiation. In addition, a newly discovered class of epigenetic regulatory proteins includes the Polycomb group (PcG) proteins and antagonists such as the histone demethylase JMJD3, both of which have recently been implicated in the control of epidermal proliferation and differentiation. The primary goal of this study is to characterize the mechanistic interactions between PcG proteins, PcG antagonists, and p63 in the control of epidermal proliferation and differentiation. To this end, we systematically depleted the expression of p63 by RNAi in order to assess resultant effects on the epidermal differentiation gene expression program. In parallel, we used chromatin immunoprecipitation (ChIP) analysis to examine p63 occupancy of differentiation gene promoters under growth and differentiation conditions. We then used ChIP analysis to assess the impacts of p63 depletion in the context of JMJD3 overexpression on chromatin regulation at loci of importance.

We found that depletion of p63 resulted in suppression of expression levels of a subset of differentiation genes. Overexpression of JMJD3 alone resulted in induction of differentiation gene expression, while concomitant depletion of p63 resulted in decreased induction of differentiation gene expression. Promoter studies of differentiation genes did not show any change in p63 promoter occupancy under proliferation and differentiation conditions. These results indicate that coordinated actions of p63 and PcG antagonists are required for proper induction of the epidermal differentiation gene program.



M. D. CASTILLO



J. K. CHEN

TUESDAY
Room D-124

8:45 A.M.

Clonal outbreak of the apicomplexan parasite *Sarcocystis neurona* in southern sea otters highlights key concepts in eukaryotic disease pathogenesis

JERED M. WENDTE, Research Scholar, Oklahoma State University Center for Veterinary Health Sciences

Preceptor: Michael E. Grigg, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Southern sea otters (*Enhydra lutris nereis*) occupy a unique ecological position as near-shore-dwelling predators of filter-feeding invertebrate prey that makes them susceptible to pathogens of terrestrial origin with the ability to be concentrated in their food source. As a federally listed threatened species, much effort has been made to understand factors leading to disease in others in order to guide conservation efforts. Gaining insight into disease processes in sea otters also provides an important model system for disease flow in the natural environment. In April 2004, an epizootic resulting in the death of a high number of southern sea otters occurred in the Estero Bay region of central California. Neurologic signs and pathologic findings were consistent with infection by the terrestrial apicomplexan parasite, *Sarcocystis neurona*. The nature of this outbreak as a sudden, dramatic increase in mortality over a short time span is suggestive of a virulent clone of *S. neurona* as the causative agent. Expansion of highly virulent clones in the occurrence of viral and bacterial disease epidemics is well documented, but this paradigm has not been established for eukaryotic pathogens. To examine whether the severity of this outbreak was the result of infection with a single clone of a eukaryotic pathogen, high-resolution microsatellite markers were designed to genotype parasite isolates. Microsatellite sequence diversity from outbreak isolates was compared against *S. neurona* isolates obtained at different locations or time periods. Size polymorphisms in the markers confirm a clonal outbreak and have important conceptual implications for eukaryotic disease pathogenesis and human health.



J. M. WENDTE



S. BEAUDRY

9:00 A.M.

Role of membrane oxidation on the functional display of *Plasmodium falciparum* erythrocyte membrane protein-1, the principal virulence factor on the surface of parasitized erythrocytes

STEVEN BEAUDRY, Research Scholar, West Virginia School of Osteopathic Medicine

Preceptor: Rick M. Fairhurst, M.D., Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Malaria, a parasitic disease transmitted by *Plasmodium falciparum*, afflicts an estimated 500 million African children annually and kills more than 1 million of them. *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) plays a critical role in the pathogenesis of severe malaria. On the surface of parasitized erythrocytes (RBCs), PfEMP-1 is concentrated on protuberances ("knobs") where it mediates adherence of parasitized RBCs to host microvessels and causes severe disease. Genetic polymorphisms that produce sickle hemoglobin S (HbS), HbC, α -thalassemia, and glucose-6-phosphate dehydrogenase deficiency are common in malaria-endemic regions of Africa, where they have been associated with protection against severe malaria and death. These polymorphisms are believed to protect against severe malaria by interfering with the normal display of PfEMP-1, thereby impairing cytoadherence interactions, but the specific mechanism is not known.

We hypothesized that membrane-bound hemichromes interfere with the trafficking and display of PfEMP-1. To test this hypothesis, we treated normal RBCs with 4 mM diethyl maleate to deplete them of reduced glutathione and "prime" them for parasite-induced hemichrome formation. We then infected these RBCs with *P. falciparum* and cultured to the mature trophozoite stage. Compared to untreated samples, parasitized DEM-treated RBCs showed significant reductions in surface PfEMP-1 detected by flow cytometry and in knob density observed by atomic force microscopy. Abnormal PfEMP-1 display on the surface of parasitized DEM-treated RBCs was associated with commensurate reductions in adherence to human microvascular endothelial cells (HMVECs) ($P < 0.0001$). When parasitized DEM-treated RBCs were cultured in the presence of cyanate, which prevents hemichrome binding to the RBC membrane, adherence to HMVECs was normal. These data suggest that hemichrome-induced changes at the inner RBC membrane are involved in abnormal PfEMP-1 display and contribute to the malaria protective effects of RBC polymorphisms.

9:15 A.M.

Toward a paratransgenic approach against visceral leishmaniasis**HEIDI HILLESLAND**, Medical Fellow, University of New Mexico School of Medicine

Mentors: Ravi Durvasula, M.D., and Ivy Hurwitz, Ph.D., University of New Mexico School of Medicine, Department of Internal Medicine

■ Leishmaniasis is a global health concern with an estimated 12 million people infected and 367 million at risk. Visceral leishmaniasis (VL) is the most devastating form of the disease and, if left untreated, has a mortality approaching 100% within two years. The greatest number of cases is reported in Bihar, India, where the causative agent is *Leishmania donovani* and the leading vector is *Phlebotomus argentipes*.

Our group is developing a novel paratransgenic strategy to control vectorial transmission of leishmaniasis. In this strategy, commensal bacteria found within the gut of *P. argentipes* are isolated and genetically altered to elaborate anti-*Leishmania* molecules. Transgenic bacteria are delivered back to the insect vector to block pathogen transmission. A critical step to this approach is the identification of commensal microorganisms within the vector. We performed an extensive analysis of the gut flora of *P. argentipes* trapped from four VL-endemic sites in Bihar and identified 28 distinct gut microorganisms. Several nonpathogenic soil bacteria that could be used in the paratransgenic system were isolated, including *Bacillus megaterium*, *Bacillus subtilis*, and *Bacillus pumilus*. These bacteria were transformed with a shuttle plasmid that expresses GFP and with three antimicrobial peptides with proven leishmaniacidal activity: mellitin, magainin, and cecropin. Protein expression from each transformant was verified by ELISA, Western analysis, and fluorescent microscopy. We are in the process of characterizing each transformant for plasmid stability and horizontal gene transfer. When fed to 4th instar *P. argentipes* larvae, we demonstrated the transstadial passage of transformed *B. pumilus* to emergent sandfly. These results suggest that the addition of the transgenic bacteria to the soil at *P. argentipes* breeding sites could be a feasible paratransgenic approach to leishmaniasis.

9:30 A.M.

HIV-1 vpr causes DNA damage in human proximal tubule cells: insights into the mechanism of HIV-associated nephropathy (HIVAN) pathogenesis**JUSTIN CHAN**, Medical Fellow, Mount Sinai School of Medicine

Mentor: Paul E. Klotman, M.D., Mount Sinai School of Medicine

■ HIV-associated nephropathy (HIVAN) is a major cause of end-stage renal disease in HIV-1 seropositive patients, particularly those of African descent. Transgenic HIV-1 gene expression in the kidney results in characteristic HIVAN pathology, including collapsing FSGS, interstitial inflammation, and tubular microcystic dilation with epithelial flattening.

Previous studies have shown that HIV-1 *nef* and *vpr* are responsible for much of HIVAN pathology. *Nef* causes podocyte dedifferentiation and proliferation, and *vpr* impairs renal tubular epithelial cell (RTEC) division, leading to hypertrophy, hyperploidy, and apoptosis. However, the mechanism for *vpr*-induced pathology is unclear. Here, we hypothesize that HIV-1 *vpr* activates the DNA damage response pathway leading to the observed renal pathology.

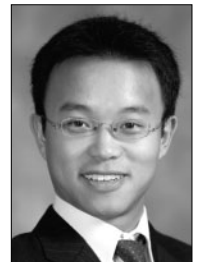
Human RTECs were transduced in vitro using a pseudotyped lentivirus vector carrying HIV-1 *vpr* and control genes. γ -H2AX immunostaining was used to measure DNA damage pathway activation both in vitro and in vivo. Reactive oxygen species (ROS) were detected by staining RTECs with 2',7'-dichlorofluorescein diacetate (H2DCFDA). In addition, we investigated the Vpr-DCAF1 (an E3 ligase substrate specificity module) interaction in RTECs by immunoprecipitation and also shRNA knockdown.

HIV-1 *vpr* expression in RTECs increased γ -H2AX levels, a marker of the DNA damage response, as seen by immunofluorescence and flow cytometry. ROS activity was also increased in *vpr*-expressing RTECs, perhaps contributing to this activation. DCAF1 knockdown in RTECs resulted in a decrease in G2 accumulation and levels of γ -H2AX when cells were transduced with *vpr*. Transgenic murine HIVAN tissue and human HIVAN tissue also showed greater γ -H2AX-positive nuclei compared to control.

This study reveals that HIV-1 *vpr* triggers the DNA damage response in RTECs, possibly due to ROS accumulation. This mechanism may explain the observed HIVAN pathology in the renal tubule compartment.



H. HILLESLAND



J. CHAN

TUESDAY
Room D-124

9:45 A.M.

The C proteins of human parainfluenza virus type 1 (HPIV1) suppress host innate immunity and apoptosis by blocking IRF and NF- κ B signaling

JIM B. BOONYARATANAKORNKIT, Research Scholar, University of California, San Francisco, School of Medicine

Preceptors: Brian R. Murphy, M.D., and Peter L. Collins, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Human parainfluenza virus type 1 (HPIV1) is an important respiratory pathogen in children and the most common cause of viral croup. We performed a microarray-based analysis of gene expression kinetics to examine how wild-type (wt) HPIV1 infection altered gene expression in human respiratory epithelial cells and what role beta interferon (IFN) played in this response. We similarly evaluated HPIV1-P(C-), a highly attenuated and apoptosis- and IFN-inducing virus that does not express any of the four C proteins, and HPIV1-C^{F170S}, another apoptosis- and IFN-inducing but less attenuated mutant that contains a single point mutation in C to examine the role of the C accessory proteins in controlling host gene expression. Mutation or deletion of the C proteins of HPIV1 permitted the activation of over 2,000 cellular genes that otherwise would be suppressed by HPIV1 infection. Cellular pathways targeted by the HPIV1 C proteins were identified and their transcriptional control was analyzed using bioinformatics. Transcription factor binding sites for IRF and NF- κ B were overrepresented in the C protein-targeted pathways. Surprisingly, the host transcriptional response to the HPIV1-P(C-) and -C^{F170S} mutants were very similar. Furthermore, both C mutant viruses, but not wt HPIV1, strongly activated IRF3 phosphorylation and I κ B degradation, which are integral steps for IRF and NF- κ B signaling. Thus, the HPIV1 C proteins profoundly suppress the host response, particularly innate immune defense, IFN production, and apoptosis, of human respiratory cells.



J. B.
BOONYARATANAKORNKIT



A. RYDER

10:00 A.M.

Analysis of respiratory syncytial virus epitope-specific CD8+ T cell receptor clonotypes

ALEX RYDER, Research Scholar, Vanderbilt University School of Medicine

Preceptor: Barney S. Graham, M.D., Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Respiratory syncytial virus (RSV) causes upper and lower respiratory tract infections in human hosts and is associated with a large disease burden in pediatric and other select populations. Natural immunity against RSV infection is not durable, but CD8+ T cells are known to be integral to the clearing of both viral infections and the associated immunopathology. The nature of the CD8+ T cell receptor (TCR) response to RSV is investigated utilizing an established hybrid murine model of epitope hierarchy capable of recognizing both the dominant H-2K^d-restricted CD8+ T cell epitope from RSV M2 protein (SYIGSINNI) and the subdominant H-2D^b-restricted CD8+ T cell epitope from M protein (NAITNAKII).

To define the relationship between epitope hierarchy and the clonotypic selection of responding CD8+ T cell receptors in this model, we utilized a new method of single-cell multiplex PCR to identify T cell receptor sequences. Preliminary data indicate that singly-sorted CD8+ tetramer-specific T cells simultaneously yield sequence information on the heterodimeric TCR- α and TCR- β chains, allowing for more in-depth analysis of TCR epitope specificities. Patterns observed between matched pairs of TCR- α and TCR- β chains can be correlated to T cell functionality and can help elucidate the phenomenon of epitope hierarchy development.

Our data indicate that the dominant M2 epitope response is associated with more public clonotype usage, while the subdominant M epitope is associated with more private clonotypes. Molecular modeling of the peptide/MHC structure suggests that the M epitope has distinct features, particularly the lysine side chain that appears to point up toward the TCR, which may explain the stochastic selection of more private clonotypes. In contrast, the M2 epitope is relatively flat and featureless, perhaps explaining the canonical selection of more public clonotypes. The functional relevance of public versus private clonotypes will be further explored.

10:30 A.M.

Investigation of determinants of susceptibility or resistance to development and progression of chronic kidney disease in inbred murine strains

TIPU S. PURI, M.D., PH.D., Early Career Awardee, The University of Chicago Pritzker School of Medicine

■ In the United States, an estimated 20 million individuals have chronic kidney disease (CKD), of which more than 8 million are considered to have moderate to severe disease. Striking differences exist in the development and rates of progression for CKD among different racial groups, undoubtedly due to environmental and genetic factors. We hypothesize that the expression of particular genes controls development and progression of kidney disease and a subset of these genes specifically determines the level of susceptibility or resistance. Development of CKD and its progression to end-stage renal disease are part of a continuum that begins with renal injury with subsequent repair processes that result in either return of function or fibrosis and loss of function. To investigate our hypothesis, we have developed a functional model of renal injury and development of CKD in mice using reversible unilateral ureteral obstruction (rUUO). In this rUUO model, C57BL/6 mice are susceptible to development of CKD, while BALB/c are resistant to CKD, illustrating fundamental genetic differences of susceptibility to injury and fibrosis. Our preliminary findings have demonstrated differences in the inflammatory responses, intrinsic renal cellular responses, and fibrosis between strains. Specific aim 1 is focused on characterizing strain-dependent differences in the inflammatory response to rUUO and the relevance to development of CKD. Specific aim 2 investigates epithelial-to-mesenchymal transition (EMT) of renal tubular epithelial cells after rUUO and a possible role for differential induction of EMT and/or associated downstream events in susceptibility or resistance to development of fibrosis and CKD in the rUUO model. Differences in TGF- β signaling pathways will be a particular focus as these have been widely implicated in EMT and fibrosis by other studies as well as our preliminary data. Specific aim 3 will use genetic approaches to investigate determinants of susceptibility and resistance to development of CKD after rUUO.

10:45 A.M.

Subverting regulation: a murine model of human food allergy

SUEJY HOBSON, Medical Fellow, David Geffen School of Medicine at UCLA

Mentor: Talal A. Chatila, M.D., David Geffen School of Medicine at UCLA

■ The maintenance of oral tolerance is contingent on the function of regulatory T cells expressing the transcription factor Foxp3, which act to prevent oral sensitization to ingested foods. Failure of regulatory T cell function due to mutations in Foxp3 is associated with the onset of severe food allergy and enteropathy in affected human subjects. However, whereas Foxp3 deficiency is extremely rare, the prevalence of food allergy is rapidly increasing, affecting large numbers of individuals in the population. We hypothesized that in food-allergic subjects, the formation of antigen-specific induced regulatory T (iT_R) cells is subverted. Whereas iT_R cells normally arise in situ from conventional CD4⁺ cells by the action of TGF- β and antigen-presenting cells of the gut, the presence of allergy-promoting cytokines alters TGF- β action to promote the formation of pathogenic T cells that promote reactivity to ingested foods.

To test this hypothesis, we established a novel murine food allergy model using a gain-of-function IL-4 receptor α chain mutant (Y709F) mice. These mice exhibit robust oral sensitization and severe anaphylactic responses to the antigen ovalbumin, identified by a significant decrease in temperature, high symptom score, and increased vascular permeability, even in the absence of adjuvants during the sensitization process. By using adoptively transferred TCR transgenic naive and regulatory T cells, we are currently examining the evolution of the eventual outcome of the regulatory T cell response in these mice. Our results will identify fundamental mechanisms by which oral tolerance is enforced and mechanisms by which its breakdown fosters human diseases, including food allergy and inflammatory bowel disease.

TUESDAY
ROOM D-124

T. S. PURI



S. HOBSON

TUESDAY
Room D-124

11:00 A.M.

Th1 effector cells convert into regulatory T cells after encountering self-antigen in vivo

DAVID CHRISTOPHER CARETTO, Medical Fellow, University of California, San Francisco, School of Medicine

Mentor: Abul K. Abbas, M.D., University of California, San Francisco

■ The immune system has the remarkable ability to simultaneously recognize and eliminate an enormous diversity of foreign antigens while remaining unresponsive to self-antigens. In response to recognition of their antigens, CD4 T cells can differentiate into several subsets, including pathogenic Th1 and Th17 effector cells and regulatory T cells (Tregs). Importantly, if Tregs are nonfunctional or absent, severe autoimmunity can occur. Our laboratory has developed a model of systemic inflammation where CD4 DO11 T cells specific for chicken ovalbumin (Ova) are transferred into lymphopenic recipient mice expressing soluble Ova protein as self-antigen (sOva-Tg Rag^{-/-} mice). After recognizing their self-antigen, these self-reactive T cells develop into effector and regulatory populations, with Th17 and Th1 effector cells being detectable prior to the appearance of Tregs, but it is unclear whether Tregs arise de novo or from previously active effector subsets.

To address this question, we have bred IFN γ -YFP reporter mice (YETI) onto the DO11 transgenic background, and these DO11 CD4 cells were cultured under Th1 polarizing conditions to generate YFP⁺ Th1 effector cells for transfer into sOva-Rag^{-/-} Tg mice. At 10 days posttransfer, T cells were harvested to assess inflammatory cytokine production and the expression of FoxP3, the transcription factor for Tregs. Surprisingly, a subset of the transferred Th1 effectors became IFN γ -YFP⁻ and expressed FoxP3. These data show that the phenotype of T cell subsets can have plasticity in vivo and support a novel concept in which effector functions may attenuate their pathogenic function through conversion into protective regulatory T cells.



D. C. CARETTO



E. M. MEOLI

11:15 A.M.

Transforming growth factor- β signaling abnormalities in central nervous system demyelinating diseases: differential SMAD7 expression in peripheral blood mononuclear cells from healthy donors and multiple sclerosis patients

ELISE M. MEOLI, Research Scholar, University of Rochester School of Medicine and Dentistry

Preceptor: Steven Jacobson, Ph.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ In vivo evidence suggests transforming growth factor- β (TGF- β) plays a key role in suppressing autoimmune reactions. One of the genes induced by TGF- β is SMAD7, which modulates TGF- β signaling in a negative feedback manner. Aberrant SMAD7 expression has been implicated in autoimmune conditions, including inflammatory bowel disease and scleroderma, and microarray data suggest that SMAD7 is differentially expressed in peripheral blood mononuclear cells (PBMCs) from multiple sclerosis (MS) patients compared to those from healthy donors. Given the evidence linking defective TGF- β signaling, aberrant SMAD7 expression, and autoimmunity, we sought to evaluate the TGF- β pathway in subsets of PBMCs from MS patients.

Using quantitative real-time PCR, we found a robust downregulation of SMAD7 mRNA expression in isolated CD4⁺ cells and total PBMCs from untreated MS patients compared with healthy controls. Expression of the TGF- β -induced cell-cycle regulator p21 was similarly found to be downregulated in MS PBMCs samples and correlated with SMAD7 expression, providing evidence to link the differential expression of SMAD7 to the TGF- β pathway.

To determine whether PBMCs from MS patients are defective in their capacity to upregulate SMAD7 expression in vitro, cells were stimulated with TGF- β after serum starvation. There was no statistically significant difference in the phosphorylation of SMAD3 as measured by Western blot or in the induction of SMAD7 mRNA in PBMCs from healthy donors and MS patients in response to TGF- β stimulation. These data suggest an extrinsic explanation for the downregulation of TGF- β -inducible genes in MS PBMCs rather than an intrinsic defect in TGF- β signaling.

These preliminary results demonstrate the differential expression of SMAD7 in MS PBMCs and suggest the possibility that these cells are exposed to less TGF- β or an inhibitor of TGF- β signaling in vivo. The possibility that dysfunctional TGF- β signaling contributes to the neuroinflammation characteristic of MS is being explored.

11:30 A.M.

Tolerogenicity of skin grafts in graftectomized tolerant animals**JOSHUA I. WEINER**, Medical Fellow, Yale School of Medicine

Mentor: David H. Sachs, M.D., Harvard Medical School

■ We have shown that tolerance to class I disparate renal allografts in miniature swine is induced by a 12-day course of cyclosporine A. This tolerance has been shown to involve T regulatory cells (Treg) and to persist for 3–4 months after graft removal. Six weeks after graftectomy, donor-type class I peptide immunization sensitized tolerant swine to future donor-type grafts, but donor skin failed to sensitize ($n=3$). In contrast, naïve animals are sensitized by both. In this study, we further investigated the tolerogenicity of skin grafts by challenging simultaneously with peptide and skin.

Miniature swine are made tolerant to class I MHC mismatched renal grafts. Graftectomy is performed after 100 days. Six weeks later, pigs are simultaneously challenged with donor-type class I peptide and donor-type skin grafts. After six more weeks, a second donor-type kidney is transplanted without immunosuppression.

Thus far, one of six animals on this protocol has been completed. In contrast to animals treated only with peptide, all of which were sensitized to second renal allografts (rejection in 4–6 days) and developed strong antidonor CTL and antidonor IgM and IgG ($n=3$), this recipient showed only a transient antidonor CTL response and no antidonor antibody production. A second donor-type class I mismatched renal allograft was accepted >90 days, and a second donor-type skin graft did not show sensitization (survived 17 days). In contrast, third-party skin was rejected in six days, indicating that hyporesponsiveness to the donor was specific.

In an animal tolerant of a class I mismatched renal allograft, which is usually sensitized by class I peptide (indirect pathway of sensitization) at six weeks after graftectomy, a simultaneous donor-mismatched skin graft appeared to prevent sensitization. These data are consistent with expansion of Treg, presumably by the direct pathway of activation, following a donor-type skin graft.

11:45 A.M.

Functional and genetic characterization of a novel human immune disorder**JEREMIAH C. DAVIS**, Research Scholar, The George Washington University School of Medicine and Health Sciences

Preceptor: Helen Su, M.D., Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Human immune disorders constitute a spectrum of functional abnormalities resulting in immunodeficiency or autoimmunity. By defining the genetic mutations responsible for these disorders, we hope to better understand how immune system homeostasis is normally regulated and how disruptions lead to disease pathogenesis. This is illustrated by the autoimmune lymphoproliferative syndrome (ALPS), in which mutations in the death receptor Fas or components cause defective lymphocyte apoptosis, leading to accumulation in lymphoid organs and autoimmunity.

We are studying a 10-year-old boy who has an unidentified immune disorder that resembles ALPS. He has lymphocytosis, lymphadenopathy, splenomegaly, autoimmune hemolytic anemia, and idiopathic thrombocytopenic purpura. However, he also has dysgammaglobulinemia, which is not characteristic of ALPS. A *WAS* variant (Ala236Thr) appeared to be noncontributory, as lyonization of maternal X chromosomes was normal by microsatellite analysis. His peripheral blood lymphocytes displayed normal or increased cell death in response to standard apoptosis stimuli: T cell receptor (TCR) restimulation, Fas, cytokine withdrawal, staurosporine, and γ - or UV irradiation. Screening of candidate molecules by Western blot revealed normal expression of caspase-8, caspase-10, c-FLIP, FADD, WASP, and Bcl-xL. T cells failed to proliferate in response to T cell receptor stimulation, as measured by CFSE dilution, and had impaired upregulation of CD69 and CD25 activation markers. Notably, ICOS, which is an important component of T cell help for antibody responses, was not induced.

Comparative genomic hybridization (CGH) microarray revealed several potential targets for further inquiry. In sum, despite his exaggerated lymphoproliferative disease in vivo, lymphocyte activation and expansion in vitro were decreased, suggesting increased production of cells with rapid turnover also contributing to immune dysregulation.



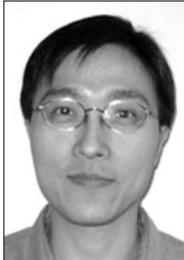
J. I. WEINER



J. C. DAVIS

TUESDAY
ROOM D-125

8:45 A.M.

BMP type I receptor inhibition reduces heterotopic ossification**PAUL B. YU, M.D., PH.D.**, Early Career Awardee, Harvard Medical School

P.B. YU



H. J. CHONG

■ Fibrodysplasia ossificans progressiva (FOP) is a congenital disorder of progressive and widespread postnatal heterotopic ossification of soft tissues, without known effective treatments. Affected individuals harbor conserved mutations in the *Acrv1* gene encoding the bone morphogenetic protein (BMP) type I receptor ALK2, which result in its constitutive activation. Here, we show that intramuscular expression of an inducible transgene encoding constitutively active ALK2 (caALK2), triggered by adenoviral gene transfer of Cre recombinase (Ad.Cre), leads to ectopic endochondral bone formation, joint fusion, and functional impairment. A selective inhibitor of BMP type I receptor kinases, LDN-193189, inhibits SMAD1/5/8 activation in tissues expressing caALK2, reducing heterotopic ossification and functional impairment. In contrast to adenovirus-induced expression of caALK2, global postnatal expression of caALK2 by itself does not lead to ectopic ossification; however, overlay of infection with control adenovirus induces ectopic bone. Corticosteroid treatment inhibits ossification in Ad.Cre-injected mutant mice, suggesting caALK2 expression and an inflammatory milieu are both required for the development of FOP lesions. This model confirms the role of dysregulated ALK2 kinase activity in the pathogenesis of FOP and suggests that small molecule inhibition of BMP type I receptor activity may be useful in treating FOP and heterotopic ossification syndromes associated with excessive BMP signaling.

9:00 A.M.

Rescuing craniofacial development in an avian model of holoprosencephaly**H. JONATHAN CHONG**, Medical Fellow, University of California, San Francisco, School of Medicine

Mentor: Ralph S. Marcucio, Ph.D., University of California, San Francisco

■ Development of the face occurs via the integrated maturation of distinct primordia. The frontonasal process and paired maxillary processes give rise to the upper jaw. Outgrowth of this region is controlled by an ectodermal signaling center named the frontonasal ectodermal zone (FEZ). This zone of ectoderm is characterized by a boundary between two distinct domains of cells that express *Sonic hedgehog* (*Shh*) and *Fibroblast growth factor 8* (*Fgf8*). Induction of the *Shh* expression domain requires SHH signaling within the developing brain; inhibition of SHH signaling in the forebrain abolishes *Shh* expression in the FEZ and leads to phenotypic changes resembling holoprosencephaly in humans. Additionally, previous studies have shown that inhibition of signaling by bone morphogenetic proteins (BMPs) also results in a downregulation of *Shh* expression in the FEZ as well as defects in craniofacial morphogenesis. Thus, communications from the developing brain and signaling by BMPs are essential for appropriate facial development. However, the exact molecular mechanisms through which the brain regulates *Shh* expression in the FEZ remain unknown.

The aim of this study was to determine the extent to which SHH or BMP signals mediate this molecular interaction between the brain and face. Inhibition of SHH signaling in the forebrain generated the expected holoprosencephalic phenotypes. Rescue was then attempted by exogenously adding either BMP or SHH protein into the head mesenchyme. Unexpectedly, activation of the BMP pathway led to a dramatic increase in cellular death in the head mesenchyme, which compounded the craniofacial phenotypes. In contrast, when the SHH pathway was activated following neural SHH blockade, a partial rescue of craniofacial morphology and ectodermal gene expression was observed. These results suggest that BMP signaling does not function downstream of neural SHH signals but that SHH signaling may instead directly regulate *Shh* expression in the FEZ.

9:15 A.M.

Interaction between Wnt and BMP signaling in joint development**JOSHUA A. GORDON**, Research Scholar, David Geffen School of Medicine at UCLA

Preceptor: Yingzi Yang, Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Joint formation and limb patterning require tightly regulated molecular cues to direct proper morphogenesis. BMP and Wnt signaling are central to these processes. Wnt signaling inhibits chondrogenesis and is required for joint formation, whereas BMP signaling is necessary for chondrocyte differentiation and inhibits joint formation. In this system, the Wnt and Bmp pathways are antagonistic to each other, yet little is known about the nature of this antagonism. Understanding the molecular mechanism of this interaction may shed light on the physiology of development and pathophysiology of joint disease. In addition, these studies may lead to novel strategies for cartilage tissue engineering.

Mutations in *Growth Differentiation Factor 5* (*GDF5*), a member of the BMP family, cause shortened limbs, altered phalange numbers, and abnormal bone and joint morphology. Using Topgal transgenic mice, an in vivo β -galactosidase reporter for Wnt signaling, it was observed that mice that were homozygous for the mutation had excessively active Wnt signaling in the developing joint. In addition, skeletal abnormalities in these same mutants were ameliorated when β -catenin was genetically removed from the developing limb. In vitro luciferase assays supported these findings in that BMP significantly reduced Topflash (Wnt) luciferase activity in both primary and 10T1/2 cells. Preliminary data also support a direct antagonistic interaction of these pathways in dorsal ventral limb patterning. We are currently examining expression patterns of joint markers in mutant mice and continue to investigate in vivo interaction of the BMP and Wnt signaling pathways.

9:30 A.M.

A novel role for TRPV4 as a modulator of interleukin-1 effects on chondrocytes**MOHAMAD HALAWI**, Medical Fellow, Duke University School of Medicine

Mentors: Farshid Guilak, Ph.D., and Wolfgang Liedtke, M.D., Ph.D., Duke University Medical Center

■ Chondrocytes, the sole resident cells in cartilage, experience dynamic osmotic changes secondary to the mechanical loading of joints. Aberrant regulatory responses by chondrocytes during this changing mechano-osmotic environment have been implicated in osteoarthritis (OA), the most common degenerative joint disease. This study examines the relationship between interleukin-1 (IL-1), a pro-inflammatory cytokine that has been implicated as a mediator of cartilage degradation in OA, and transient receptor vallerinoid 4 (TRPV4), an osmotically sensitive ion channel, in order to provide new insights into the pathophysiology of OA.

Interactions between IL-1 and TRPV4 were investigated by examining their effects on early signaling events (intracellular Ca^{2+} mobilization; $[\text{Ca}^{2+}]_i$) and downstream activity (specific activity of matrix metalloproteinases; MMPs) in primary porcine chondrocytes treated under either iso-osmotic (380 mOsm) or hypo-osmotic (280 mOsm) conditions. $[\text{Ca}^{2+}]_i$ was measured using a high-throughput fluorometric imaging plate reader (FLIPR). Total specific MMP activity was measured in culture media using a fluorescence-based assay. While TRPV4-mediated $[\text{Ca}^{2+}]_i$ was significantly higher under hypo-osmotic than iso-osmotic conditions ($P < 0.001$), this osmotically mediated sensitization of TRPV4 was inhibited by IL-1 only under hypo-osmotic conditions ($P < 0.05$). Furthermore, while chondrocytes cultured under iso-osmotic conditions in the presence of IL-1 showed a significant dose-dependent increase in specific MMP activity, chondrocytes cultured with IL-1 under either hypo-osmotic conditions or in the presence of $4\alpha\text{PDD}$, a selective TRPV4 agonist, demonstrated markedly decreased specific MMP activity ($P < 0.05$). The inhibition of IL-1-induced MMP activity by TRPV4 activation was evident regardless of culture media osmolarity.

These findings suggest an important role for TRPV4 as a modulator of IL-1 effects in chondrocytes. Additional work is needed to elucidate the molecular events that follow the exposure of chondrocytes to IL-1 and that lead to inhibition of TRPV4. Further work is also needed to unravel the molecular cascade by which TRPV4 inhibits IL-1-mediated MMP production.



J.A. GORDON



M. HALAWI

TUESDAY
ROOM D-125

9:45 A.M.

Novel inflammatory gene expression and cytokine response in the MRL/MpJ mouse following a closed knee fracture**JOHN STRUDWICK LEWIS JR.**, Medical Fellow, Duke University School of Medicine

Mentor: Steven A. Olson, M.D., Duke University School of Medicine

■ Posttraumatic arthritis (PTA) is a frequent long-term complication of articular fractures. In a mouse model of closed articular fracture, C57BL/6 mice developed PTA, whereas a second strain of mice, MRL/MpJ, did not. These healing characteristics have been attributed to decreased levels of inducible pro-inflammatory cytokines in MRL/MpJ mice. The objective of this investigation was to determine whether MRL/MpJ mice exhibit a reduced inflammatory response following closed articular fracture compared to C57BL/6 mice. Intra-articular fractures of the tibial plateau were created in C57BL/6 ($n=30$) and MRL/MpJ ($n=30$) mice. Six mice from each strain were sacrificed at prefracture and at 0, 1, 3, 5, and 7 days postfracture. Gene expression in synovial tissue was assessed using a mouse inflammatory cytokine RT-PCR 84-gene array. Local and systemic levels of cytokines were measured in synovial fluid and serum, and the presence of inflammatory mediators in tissue was examined by immunohistochemistry.

For C57BL/6 mice, 54 of the 84 genes evaluated were upregulated following fracture versus 33 genes for MRL/MpJ mice. C57BL/6 mice showed a 700-fold increase in IL-1 β expression within 4 hours of fracture compared to a 70-fold peak for MRL/MpJ mice. IL-1 β concentration was significantly increased in the synovial fluid following fracture for both strains. The fold change compared to control was 7-fold for C57BL/6 mice and only 2-fold for MRL/MpJ mice. C57BL/6 mice also demonstrated significantly higher systemic serum levels of IL-1 β ($P=0.002$). Preliminary immunohistochemistry has shown significantly increased numbers of activated macrophages and increased IL-1 β in the articular cartilage, menisci, and synovial tissue of C57BL/6 mice as compared to MRL/MpJ mice.

Our results demonstrate that MRL/MpJ mice show significantly reduced intra-articular and systemic inflammation with decreased IL-1 β gene expression and protein levels following articular fracture. Identifying the role of these pro-inflammatory cytokines following fracture may help elucidate the mechanisms involved in the progression of PTA.

10:00 A.M.

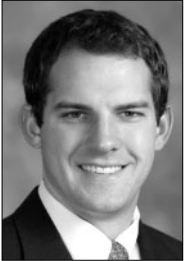
Activation of liver X receptor promotes vascular cell calcification**JEFFREY J. HSU**, Medical Fellow, David Geffen School of Medicine at UCLA

Mentors: Linda L. Demer, M.D., Ph.D., David Geffen School of Medicine at UCLA, and Peter Tontonoz, M.D., Ph.D., Howard Hughes Medical Institute, David Geffen School of Medicine at UCLA

■ Vascular calcification is prevalent in atherosclerotic lesions and correlates with an increased risk of cardiovascular events, such as myocardial infarction, stroke, and death. Recent studies have demonstrated that activation of the nuclear receptor liver X receptor (LXR) reduces atherosclerotic lesion size in an animal model by promoting cholesterol efflux from macrophages and decreasing inflammatory gene expression. Given the association of atherosclerosis and vascular calcification, we examined whether LXR activation also reduces vascular cell calcification. Primary aortic cells from wild-type (WT) and *Lxr β ^{-/-}* mice were induced to calcify by using forskolin, an activator of the protein kinase A (PKA) pathway and known stimulator of vascular cell calcification. The effects of LXR activation on PKA-induced matrix mineralization and osteogenic markers were tested by cotreating the cells with T0901317, a synthetic LXR agonist.

Results showed that T0901317 augmented forskolin-induced alkaline phosphatase (ALP) activity and matrix mineral incorporation in WT cells, but not in *Lxr β ^{-/-}* cells, while T0901317 alone had no effect. Treatment with GW3965 or 25-hydroxycholesterol, other known LXR agonists, produced similar results. Inhibition of ALP, the phosphate cotransporter Pit-1, or Rho-associated kinase (ROCK) attenuated the effects of T0901317 on ALP activity and matrix mineralization. However, inhibition of PKA had no significant effect on the actions of T0901317. Short-term treatment with T0901317 augmented the PKA-induced expression of Pit-1, a positive regulator of mineralization, in WT cells, but not in *Lxr β ^{-/-}* cells. Long-term treatment with T0901317 attenuated PKA-induced expression of two known inhibitors of matrix mineralization, osteopontin and ectonucleotide pyrophosphate/phosphodiesterase-1 (Enpp1).

Contrary to our initial hypothesis, these results suggest that activation of LXR augments PKA-induced matrix mineralization in vascular cells via both direct and indirect LXR-dependent pathways. The effects of T0901317 appear to be ROCK-dependent, but downstream of PKA.



J. S. LEWIS JR.



J. J. HSU

10:30 A.M.

Transcriptional profile of genes involved in axonal transport correlated to UHR-SD OCT from a mouse model of optic neuritis

ARI GREEN, M.D., Early Career Awardee, University of California, San Francisco, School of Medicine

■ The objective of this work was to evaluate transcriptional regulation of genes involved in axoplasmic transport in mice with spontaneous optic neuritis and signal change on optical coherence tomography (OCT).

OCT measures the backscatter of low-coherence infrared light from retinal structures to provide in vivo imaging. Swelling of the inner retina can be seen in human subjects with acute optic neuritis, but the cause of this change is currently unknown. We have performed the first OCT imaging from a rodent model of retrobulbar optic neuritis demonstrating early inner retinal injury.

Six- to eight-week-old MOG-TCR transgenic mice catalyzed with pertussis toxin to develop optic neuritis have been sequentially imaged using ultra-high resolution spectral domain OCT (UHR-SD-OCT) and compared to wild-type BL-6 mice of the same age. Following the appearance of signal change in the inner retinal band (>10% change from baseline), animals are sacrificed. mRNA transcript levels for proteins involved in axonal transport (kinesins, stathmin, tubulin) have been evaluated from a laser capture microdissection (LCM)-enriched population of retinal ganglion cells (RGCs) using qRT-PCR.

Imaging changes in the inner retinal band have been identified that indicate early swelling of the inner retina (days 6–10) followed by subsequent cell/tissue loss (after day 12). LCM-enriched populations of cells have yielded sufficient RNA for amplification and quantification. Quantitative evaluation of transcriptional levels is ongoing. Further analysis of OCT images is still underway to evaluate whether earlier changes may be detectable. Future work will include histological preparations of retinas with immunohistochemical stains for relevant proteins.

This work could help identify whether swelling of the inner retina with retrobulbar optic neuritis is a consequence of failure in axonal transport and whether this process is transcriptionally regulated.

10:45 A.M.

Contributions of presynaptic calcium dynamics at functionally divergent release sites to target-cell-dependent plasticity within the auditory nerve-cochlear nucleus circuit

STEVE KHACHI, Research Scholar, Des Moines University, College of Osteopathic Medicine

Preceptor: **Stephan D. Brenowitz, Ph.D.**, National Institute on Deafness and Other Communication Disorders, National Institutes of Health

■ The auditory system provides an excellent opportunity to study how information is processed and transformed by neuronal circuits. Auditory information is transduced in the cochlea and enters the brain via the auditory nerve (AN), which then projects to the cochlear nucleus (CN). Individual axons of the AN form synapses onto different types of neurons in the CN that exhibit distinct responses to sounds. Although these cells receive the same activity pattern of synaptic inputs, their responses differ in part because of distinct specialized synaptic mechanisms. Furthermore, in response to AN input, CN neurons exhibit several forms of activity-dependent short-term plasticity such as facilitation and depression in a target-dependent manner. However, the mechanisms that enable an axon to independently regulate release properties at different presynaptic boutons are not yet well-understood.

Calcium signals in presynaptic boutons regulate many forms of short-term plasticity, and some of the differences in synaptic plasticity at distinct targets are accounted for by differences in presynaptic calcium transients. Therefore, presynaptic calcium measurements are critical for studying synaptic mechanisms, but this has been done in few preparations and not yet at the AN terminals. After surgically injecting fluorescent calcium indicators into the cochlea of mice, we will use two-photon laser scanning fluorescence microscopy to measure presynaptic calcium transients in individual presynaptic AN terminals. The imaging studies, combined with electrophysiological recordings from postsynaptic neuronal targets, will reveal mechanisms that underlie target-specific properties of synaptic transmission in the CN. These studies will provide information about how individual axons regulate neurotransmitter release and short-term plasticity at individual boutons that contact functionally divergent postsynaptic targets. These findings will ultimately contribute to our understanding of the roles of synaptic transmission and cellular mechanisms of hearing disorders such as congenital deafness, noise-induced hearing loss, tinnitus, and presbycusis.

TUESDAY
ROOM D-125

A. GREEN



S. KHACHI

TUESDAY
Room D-125

11:00 A.M.

**Reversal of the serotonin
“reuptake” transporter**

SEAN McEVoy, Medical Fellow, Yale School of Medicine

Mentor: George Richerson, M.D., Ph.D., Yale School of Medicine

■ The serotonin transporter (SERT) has been associated with reuptake of neurotransmitter from the synaptic space. This transportation of serotonin is commonly conceptualized as a unidirectional event involving the movement of serotonin from the synaptic space into the presynaptic bouton. However, previous work in our lab has shown that the GABA transporter (GAT) is capable of reversing direction under physiological conditions and transporting GABA from the presynaptic bouton into the synaptic space. Our study investigates the possibility that SERT is capable of reversing direction as well. To assay for the reversal of SERT, we studied the respiratory bursting in brainstem slices of mice, which express tetanus toxin light chain in serotonin neurons. This toxin prevents fusion of vesicles with the plasma membrane and the release of their contents into the synapse. Though vesicular release of serotonin is blocked in these animals, they do not show the respiratory phenotype that we have observed in other animals where serotonin is absent. We suspect that serotonin is released in these transgenic animals through the reversal of SERT, thus accounting for the normal respiratory phenotype. We are testing this hypothesis by bathing the brainstem slices in artificial cerebrospinal fluid, which contains 500 nM citalopram, an inhibitor of SERT, while recording respiratory bursting from the hypoglossal nerve. We expect that if SERT is reversing its transport of serotonin, it will be unable to do so when inhibited by citalopram. This will lead to the slices showing a similar respiratory phenotype to mice lacking serotonergic stimulation.



S. McEVoy



S. TOPRANI

11:15 A.M.

**Mechanisms of seizure suppression by
low-frequency electrical stimulation of the
fimbria-fornix-hippocampal commissures
in combined hippocampus-entorhinal
cortex slices in rats**

SHEELA TOPRANI, Medical Fellow, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentors: Dominique Durand, Ph.D., Case Western Reserve University, and Imad Najm, M.D., Cleveland Clinic Foundation

■ Safe and effective treatment of mesial temporal lobe epilepsy (MTLE) is a medical challenge that may be ameliorated by low-frequency electrical stimulation (LFS). A novel LFS paradigm is presented that targets the fimbria-fornix hippocampal commissure (FFHC), a major efferent and afferent pathway of the hippocampus. The objectives of this study are to evaluate the efficacy of this LFS paradigm in suppressing hippocampal seizures and to understand the mechanism of its effect.

Spontaneous epileptiform activity is induced in 750- μ m-thick transverse rat brain slices using 4-aminopyridine (4-AP). Field responses are measured in CA3 and CA1 hippocampal subfields bilaterally. Electrical stimulation (1 Hz) is applied to the FFHC and seizure activity before, during, and after LFS is compared. This procedure is repeated with the addition of GABA_A and GABA_B modulators in order to evaluate the contribution of GABA to the mechanism of LFS.

Percentage of seizure time during LFS of the FFHC was significantly suppressed ($0.86\% \pm 1.8\%$) compared to that preceding stimulation ($28.2\% \pm 13\%$) (paired $t(9) = 6.2518$, $P = 0.0001$). Bilateral seizure suppression could be achieved as well with L&R seizure time before LFS of $18.02\% \pm 3.5$ and during LFS of $0.0\% \pm 0.0\%$ (paired $t(4) = 14.6$, $P < 0.0001$). The LFS paradigm is effective in the presence of GABA_A blockade during which seizure time drops from $35.7\% \pm 9\%$ to $0.4\% \pm 1.3\%$ due to LFS (paired $t(8) = 10.8$, $P < 0.0001$), but less effective during GABA_B blockade such that seizure time drops from $10.54\% \pm 2.98\%$ to $5.95\% \pm 1.92\%$ due to LFS (paired $t(19) = 6.24$, $P < 0.0001$).

These data show that LFS of the FFHC effectively suppresses hippocampal seizures in this model and that GABA_B contributes to its mechanism.

11:30 A.M.

Testing connections between steroidogenesis and neurodegeneration in Niemann-Pick disease type C**JENNIFER HONG**, Medical Fellow, Stanford University School of Medicine

Mentor: Matthew P. Scott, Ph.D., Howard Hughes Medical Institute, Stanford University School of Medicine

■ Recent studies suggest that steroids are important in certain types of neurodegenerative disease. Niemann-Pick disease type C (NP-C) is an autosomal recessive lysosomal storage disorder characterized by cholesterol accumulation in the lungs, liver, spleen, and brain. Mutations in the gene *NPC1*, which encodes a highly conserved 13-pass transmembrane cholesterol-binding protein, account for 95% of the cases of NP-C. NP-C is lethal by adolescence, and no effective treatment exists. A hallmark of NP-C pathology is selective death of Purkinje neurons in the cerebellum. Purkinje neurons produce neurosteroids, which appear to be reduced in the brains of *Npc1* mutant mice; giving exogenous neurosteroids to these mice rescues some Purkinje death. The cellular mechanisms that underlie these changes are unknown. Steroids are synthesized from sterol precursors in multiple cellular compartments, and the failure of sterol trafficking in *npc1*^{-/-} cells may reduce steroid levels. We propose to test whether altered *Npc1* function affects steroidogenesis using a tetracycline-regulated shRNA against endogenous *Npc1*. In contrast to *npc1*^{-/-} mice, the shRNA system will knock down *Npc1* from specific cell types, in various degrees and at specific times.

In an *in vitro* setting, a Y-1 steroidogenic cell line stably transfected with our shRNA against *Npc1* shows decreased progesterone synthesis relative to wild-type Y-1 cells. Surprisingly, pregnenolone synthesis appears to be increased relative to wild-type Y-1 cells, suggesting that there may be a trafficking defect downstream of pregnenolone synthesis in mitochondria. We are currently characterizing shRNA activity *in vivo* in a transgenic mouse line. In further experiments, we will determine whether *Npc1* acts cell-autonomously in Purkinje neurons with respect to steroidogenesis, and if so, whether perturbations in steroidogenesis affect Purkinje survival.

Our preliminary data indicate that *Npc1* may play a role in trafficking sterol precursors of steroids downstream of pregnenolone synthesis in mitochondria.

11:45 A.M.

Characterizing blood-brain barrier disruption through fluid attenuated inversion recovery (FLAIR) imaging with matrix metalloproteinase levels in an animal model of middle cerebral artery occlusion**AYUSH BATRA**, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Preceptor: Steven Warach, M.D., Ph.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ Matrix metalloproteinases (MMPs) have been studied extensively in ischemic injury due to their prominent role in blood-brain barrier (BBB) disruption and hemorrhage. However, poor understanding of MMP activity and the relationship of tissue to blood levels creates obstacles for clinically implementing MMP-inhibitor therapy in the setting of acute stroke. Our goal is to understand the relationship between tissue and blood levels of MMP-2 and MMP-9 at multiple time points following experimental stroke and to associate these levels with evidence of BBB disruption on FLAIR imaging. We hypothesize that blood and tissue levels of MMPs will predict the severity and extent of BBB disruption.

Spontaneously hypertensive (SHR) rats underwent 48 minutes of right middle cerebral artery (MCA) occlusion with a nylon suture followed by reperfusion. Animals were studied at 1, 24, and 48 hours post reperfusion ($n=6$ per time point), with comparison to sham and control groups ($n=6$). FLAIR imaging was performed just prior to and 10 minutes following gadolinium injection (0.2 ml at 0.5 mol/L, *i.v.*) to assess BBB status. Blood and tissue samples were collected following imaging. MMP activity was assessed through gelatin zymography, and total protein content was measured through Western blotting.

Preliminary results indicate that MMP-9 activity levels as measured by zymography are increased acutely ($P<0.05$) but resolve to baseline sham control levels at 24 and 48 hours. Ventricular enhancement was observed at the acute time point but was not evident at 24 and 48 hours ($P<0.01$). Additionally, parenchymal enhancement in the region of the right MCA territory was present only at 48 hours ($P<0.01$). These results suggest a time-dependent association between blood MMP levels and acute BBB disruption, supporting the clinical use of MMPs as acute biomarkers for stroke. A comprehensive comparison of tissue and blood MMP levels and evidence of BBB disruption on FLAIR is currently underway.



J. HONG



A. BATRA

8:45 A.M.

Effector cell development and lineage commitment in allergic inflammation**FRED H. HSIEH, M.D.**, Early Career Awardee, Cleveland Clinic Foundation

F. H. HSIEH

■ Asthma is a chronic disease characterized by inflammation and airway remodeling with microvasculature changes. The developmental programs of hematopoietic cells and endothelium may be closely linked, though the existence of hemangioblast-like progenitors in postnatal life remains controversial and their role in human disease is not fully articulated. Mast cells (MCs) play a critical role in the pathogenesis of airway inflammation, can be derived from multipotent hematopoietic progenitors, and circulate as immature progenitors prior to completing their differentiation peripherally in the tissues. We hypothesized that human MCs and endothelial cells share a common progenitor and that this progenitor may traffic to the lung in asthmatic patients, where the lineage commitment of these cells may be directed by specific tissue micro-environmental factors.

Clonal endothelial progenitor cells (EPCs) derived from cord blood after limiting dilution analysis were identified by cell-surface phenotype (CD31+/CD34+/CD117+/CD133+/CD144+/VEGR2+), positive acetylated-LDL and *Ulex europaeus* Agglutinin-I staining, and in vitro vascular tube assay. After transfer into stem cell factor (SCF)-dependent culture, by 6 weeks, EPCs were uniformly metachromatic by toluidine blue staining and were <90% positive for both MC-specific proteases tryptase and chymase. Using an in vivo animal model, we mixed clonal single-colony human EPCs with unpolymerized matrigel and injected them subcutaneously into NOD/SCID mice. After 4 weeks, MCs could be identified in the matrigel plug by double immunostaining for both toluidine blue (identifying all MCs) and human-specific anti-tryptase or anti-chymase immunostaining, suggesting that human MCs could be derived from human EPCs in vivo. EPCs were then derived from the peripheral blood of normal and asthmatic human donors and cultured ex vivo in SCF-dependent conditions. EPCs derived from asthmatic subjects had significantly increased transdifferentiation capacity to MCs compared to normals.

With these experiments, we have identified a previously unrecognized connection between MCs and endothelial cells and propose a potentially novel origin of tissue of MCs. Future experiments will focus on the mechanisms by which these circulating multipotent progenitors may contribute to both the inflammation and airway remodeling seen in asthma.



A. CZECHOWICZ

9:00 A.M.

Niche recycling through division-independent egress of hematopoietic stem cells**AGNIESZKA CZECHOWICZ**, Medical Fellow, Stanford University School of Medicine

Mentors: Irving L. Weissman, M.D., Stanford University School of Medicine, and Deepta Bhattacharya, Ph.D., Washington University School of Medicine

■ Hematopoietic stem cells (HSCs) are used therapeutically in bone marrow/hematopoietic stem cell transplantation (BMT/HSCT) to correct hematolymphoid abnormalities, as their ability to self-renew and differentiate enables them to generate a new and complete hematolymphoid system. Currently, toxic conditioning regimens are necessary for HSC engraftment, and the risks associated with these regimens prevent widespread use of BMT. We have shown that one barrier to HSCT is the limited HSC niches that are available at any one point in time. HSCs migrate from the bone marrow under physiological conditions, as evidenced by the small number of HSCs found in the blood and lymph at any particular time in unmanipulated mice. Through bromodeoxyuridine labeling experiments, we provide evidence that HSCs egress from their supportive niches in the bone marrow to the blood independently of cell division. This division-independent migration of HSCs leads to the transient vacancy of such niches, and transplantation of an excess of donor HSCs can saturate these empty niches. Within 24 hours, niche availability resets to the levels observed prior to donor HSC transplantation, consistent with the observation that physiological HSC egress is a continuous process. Transplanted HSCs engraft at similar levels in recipients expressing a *Bcl2* transgene as in wild-type mice, suggesting that apoptosis of occupying HSCs is not a major process by which niches are emptied. These data, which specifically characterize the behavior of HSCs rather than that of unfractionated marrow, provide insight into how HSC replacement can occur despite the residence of endogenous HSCs in discrete physical niches. In addition, they suggest strategies for therapeutic intervention that capitalize on this process. Specifically, we show that through repeat rounds of HSC transplantation we can achieve high levels of stable HSC engraftment without any toxic conditioning.

9:15 A.M.

Assessing plasticity: the populations responsible for epithelial engraftment of marrow-derived cells**JUSTIN BRENT COHEN**, Medical Fellow, Yale School of Medicine

Mentor: Diane S. Krause, M.D., Ph.D., Yale School of Medicine

■ Bone-marrow-derived cells (BMDCs) have significant plasticity, allowing them to give rise to various nonhematopoietic cell types, including epithelial cells of the lung, liver, gut, and skin. These findings offer tremendous possibilities for the use of cell therapy to treat tissue injury and disease. However, the specific populations of cells responsible for this phenomenon are still unclear, with considerable controversy over the mechanism of this transformation.

In this study, we sought to compare the epithelial engraftment ability of two populations enriched for hematopoietic stem cells, specifically the fractionated, lineage-depleted, and homed subset (FLH) and the c-Kit⁺Lineage⁻Sca-1⁺ sorted population (KLS), to whole bone marrow (WBM). Plasticity capability was assessed by examining the engraftment of type II (T2) pneumocytes in the lung following sex-mismatched bone marrow transplantation in mice. The recipient mice were knock-outs of surfactant protein C (SPC), a T2-specific protein, thereby allowing detection of the transplanted wild-type cells using Y-FISH and immunofluorescence for SPC on paraffin sections and cytopins. Additionally, RT-PCR for SPC transcripts provided a sensitive method of detecting engraftment of these BMDCs. Our preliminary data suggest that the FLH population does not engraft significantly better than control WBM into either the hematopoietic system or the lung. Despite our effective detection techniques, only exceedingly rare donor-derived T2 cells could be found by both microscopy and RT-PCR. The results of the KLS population are pending, but I predict that this population will be similar to the FLH cells and demonstrate very low levels of engraftment and conversion to epithelial lineages of the lung.

These data suggest that rare incidental entrapment of transplanted BMDCs might be responsible for the presence of rare donor-derived epithelial cells. It is likely that another resident BMDC population such as mesenchymal stem cells is primarily responsible for epithelial engraftment.

9:30 A.M.

Sunitinib facilitates bone marrow engraftment through c-kit**NATASHA FEWKES**, Research Scholar, Oregon Health and Science University School of Medicine

Preceptor: Crystal L. Mackall, M.D., National Cancer Institute, National Institutes of Health

■ Bone marrow transplant (BMT) resulting in stable mixed chimerism is likely to provide clinical benefit when it is undertaken for benign diseases such as sickle cell anemia. However, BMT is often avoided in this patient population because of the morbidity associated with current ablative and nonmyeloablative conditioning regimens. We sought to provide an entirely nonmyeloablative, noncytotoxic approach to achieve stable mixed chimerism in patients with benign disease. Sunitinib, an FDA-approved tyrosine kinase inhibitor with few immune effects, inhibits signaling through c-kit, Flt3, PDGFR, and VEGFR. Notably, c-kit and Flt3 are important for hematopoiesis. We hypothesized that treating BMT recipients with Sunitinib would give donor cells a competitive advantage and allow for increased engraftment. Sunitinib treatment of RAG^{-/-} recipients (60 mg/kg/day on days -3, -2, -1, and 0) followed by transfer of 5×10^6 wild-type BM cells on days 0 and 1 enhanced HSC BM engraftment ($65.5 \pm 35 \times 10^4$ vs. $5 \pm 10 \times 10^4$ cells, $P=0.03$) in Sunitinib vs. vehicle-treated recipients. To investigate the mechanism by which Sunitinib allows for increased engraftment, we transplanted mice that were deficient in c-kit by using the above regimen. Although these mice engraft more easily even without any conditioning, preliminary data show that the effects of Sunitinib are diminished ($17.6 \pm 9\%$, $n=5$ vs. $14.2 \pm 4\%$, $n=4$ donor chimerism $P=0.24$) in Sunitinib versus vehicle-treated c-kit^{-/-} recipients. Given that c-kit-independent effects of Sunitinib do not enhance engraftment in this setting, these results suggest that the effects observed are indeed due to inhibition of c-kit signaling by Sunitinib.



J. B. COHEN



N. FEWKES

TUESDAY
AUDITORIUM

9:45 A.M.

Lentiviral vectors with leukocyte integrin CD11b promoter leads to efficient transduction of canine leukocyte adhesion deficiency CD34+ cells**CEDAR J. FOWLER**, Research Scholar, Tufts University School of Medicine

Preceptor: Dennis Hickstein, M.D., National Cancer Institute, National Institutes of Health

■ When compared to retroviral and lentiviral vectors utilizing viral promoter/enhancers, vectors with internal cellular promoters provide the potential to achieve efficient transduction of hematopoietic cells while reducing the risk of genotoxicity. The optimal approach would use promoters that are not only tightly regulated, but also specific for the target cell type. We tested the SIN lentiviral vector pRRLSIN.cPPT.cCD18 with the human leukocyte integrin CD11a or CD11b promoters to transfer canine CD18 cDNA into CD34+ bone marrow cells from dogs with canine leukocyte adhesion deficiency (CLAD). In CLAD, mutations in CD18 result in the inability to express CD11/CD18 heterodimers on the cell surface of leukocytes; rescue with CD18 results in CD11/CD18 surface expression. The CD11a and CD11b subunits are expressed exclusively on leukocytes; CD11a is expressed primarily on lymphocytes, whereas CD11b is expressed primarily on neutrophils.

We used a third-generation lentivirus vector system with 10 different upstream fragments from the human CD11a and CD11b promoters to express canine CD18 in CLAD CD34+ cells in vitro: the human CD11a promoters ranged from 356 bp to 1.7 kb, and the human CD11b promoters ranged from 388 bp to 1.6 kb. All promoters included the transcription start sites. Vectors were titrated on an LAD EBV B cell line that lacks endogenous CD18. CLAD CD34+ cells were transduced at two different MOIs (10 and 100) for each vector and analyzed by flow cytometry for CD18 expression 4 days following transduction. None of the five human CD11a promoters resulted in >10% CD18+ cells measured 4 days after transduction. This was the highest percentage of any of the CD11b promoter inserts tested. These results support the use of SIN lentiviral vectors with the 639-bp human CD11b promoter to express canine CD18 in animals with CLAD.



C. J. FOWLER



J. PEARL

10:00 A.M.

The immunogenic properties of human embryonic stem cells**JEREMY PEARL**, Medical Fellow, Stanford University School of Medicine

Mentors: Mark M. Davis, Ph.D., and Joseph C. Wu, M.D., Ph.D., Stanford University School of Medicine

■ Human embryonic stem cells (hESCs) hold promise as an unlimited source of cells for therapeutic transplantation. However, the immunological properties of hESCs in a human allogeneic transplantation setting are unknown. We investigated the immunological phenotype of hESCs and the changes that occur upon interaction with allogeneic human lymphocytes in vivo and then developed an immunosuppressive regimen capable of inducing long-term acceptance of hESCs.

To characterize the in vitro immunological phenotype of hESCs, we performed FACS analysis. hESCs expressed class-I MHC, β -2 microglobulin, ICAM-1, and LFA-3 antigens, but they were negative for MHC-II, CD40, B7-1, B7-2, and B7-H1 antigens. IFN- γ exposure stimulated hESC derivatives to express B7-H1 from differentiation day 14 and MHC-II at day 28.

To study the effects of human lymphocytes interacting with hESCs in vivo, we created a "humanized" mouse model by reconstituting immunodeficient NOD-scid Il2 γ ^{null} mice with human peripheral blood mononuclear cells (hPBMCs). hESCs were then injected into the spleen of reconstituted ("humanized") and nonreconstituted mice. At 4 weeks post-hESC transplantation, hESC-derived intrasplenic teratomas were harvested and demonstrated robust upregulation of MHC-I, MHC-II, β -2 microglobulin, ICAM-1, and B7-H1 in hPBMC-reconstituted mice as compared to nonreconstituted mice. Next, we tested whether treatment with Tacrolimus, Rapamycin, or anti-CD40L/ anti-LFA-1/CTLA4Ig could induce long-term acceptance of ESCs. We found that Tacrolimus and Rapamycin only slightly prolonged ESC survival, whereas combined treatment with the anti-CD40L/ anti-LFA-1/CTLA4Ig generated long-term ESC graft acceptance.

This is the first investigation of the immunological phenotype of hESCs in a clinically relevant human allogeneic transplantation model. Upon interaction with allogeneic lymphocytes in vivo, hESC derivatives upregulate MHC and cosignaling molecules. These data suggest that the immunogenicity of hESC derivatives progressively increases following allogeneic transplantation. Additionally, we identified an immunosuppressant regimen capable of permitting long-term engraftment of hESCs.

10:30 A.M.

Association of genome-wide DNA copy number aberrations and gene expression in metastatic oral squamous cell carcinoma

EDUARDO MÉNDEZ, M.D., Early Career Awardee, University of Washington Medical Center

■ Dysregulation of gene expression resulting from DNA copy number aberrations (CNAs) is believed to be a driving factor of tumor initiation and progression. High-resolution genome-wide assessments of the association of single nucleotide polymorphism (SNP) array-based CNAs with gene expression at the same loci have recently become possible. The purpose of this study was to examine the association of CNAs and gene expression in metastatic oral squamous cell carcinoma (OSCC). Tumor cells from lymph node metastases of 20 OSCC patients were harvested using laser capture microdissection (LCM). DNA and RNA were purified simultaneously from the same LCM-harvested tumor cells and interrogated with Affymetrix 250K Nsp SNP arrays and U133 Plus 2.0 expression arrays, respectively. DNA copy numbers were inferred from SNP using the fused-lasso algorithm. For each gene expression probe set, its DNA copy number in one sample was calculated as the average DNA copy number of the SNPs within its 250-Kb genome neighborhood. For the 62,828 probe sets on the expression array, 62,264 probe sets contained at least one SNP within their 1-Mb genome neighborhoods. After filtering out probe sets that had 1) no detectable expression; 2) had an interquartile range of variation of <0.1; 3) had multiple sequence alignments; and 4) were in X, Y, or mitochondrial locations, 23,484 probe sets were left for further analysis. The association between CNA and corresponding RNA expression levels for each gene was then examined through univariate linear regression models. A significant association between CNA and gene expression changes was found for 30.5%. Specifically, with a *P* value cutoff at 0.0001, 360 genes showed positive relationships between their CNA and RNA expression levels. The genes *NTS*, *COL10A1*, *PLGA1*, *GHR*, *LTA4H*, and *CCND2* had the most significant association between DNA copy number and gene expression. The top three molecular functions of these 360 genes as determined by Ingenuity Pathway Analysis are cell cycle, DNA replication, and recombination and repair. Further investigation of all of these genes as potential driving factors for OSCC initiation and progression is warranted.

10:45 A.M.

The search for endogenous regulators of transient receptor potential vanilloid 1

ASAFF HAREL, Medical Fellow, University of Pittsburgh School of Medicine

Mentor: Joseph C. Glorioso, Ph.D., University of Pittsburgh School of Medicine

■ Transient receptor potential vanilloid 1 (TRPV1), originally discovered as the receptor for capsaicin, was found to be a pronociceptive cation channel that preferentially conducts calcium. TRPV1 is responsive to a number of physical and chemical stimuli, and activation leads to hyperalgesia, characterized by increased painful perception of noxious stimuli at the site of injury. While much is known about endogenous stimuli that activate the channel, relatively little is known about pathways that antagonize TRPV1.

We have devised a herpes simplex virus-1 (HSV-1) replication-based method to screen a neuronal cDNA library for gene products that may interfere with capsaicin-mediated TRPV1 activation. In cell culture, activation of exogenous TRPV1 by treatment with a high concentration of capsaicin leads to calcium overload and resultant osmotic cell death. Potential interference with this activation by the presence of a gene product derived from a cDNA would allow the cell to escape death, thereby permitting the virus to replicate and amplify its genome. An HSV-1 vector was manipulated to contain the TRPV1 coding sequence in place of the thymidine kinase coding sequence and an hCMV promoter followed by a Gateway recombination cassette (Invitrogen) in place of the internal repeat region. The Gateway cassette provides a means for efficient recombination of the cDNA library into the HSV-1 vector, thereby allowing expression of both TRPV1 and a cDNA upon infection of cells with this virus. We are presently characterizing the viral vector and will use it to screen for negative modulators of TRPV1 in the near future.

The identification of novel negative modulators of TRPV1 and the characterization of their mode of action should provide information about the role of TRPV1 in pain signaling, and by extension, suggest novel approaches for the treatment of primary hyperalgesia.

TUESDAY
AUDITORIUM



E. MÉNDEZ



A. HAREL

TUESDAY
AUDITORIUM

11:00 A.M.

Genotype-phenotype correlations in patients with holoprosencephaly and alterations in *TG-Interacting Factor*

AMELIA KEATON, Research Scholar, University of South Carolina School of Medicine

Preceptor: Maximilian Muenke, M.D., National Human Genome Research Institute, National Institutes of Health

■ Holoprosencephaly (HPE) is the most common malformation of the developing forebrain and results from failed hemispheric cleavage. Even within the same family, clinical manifestations have a range of severity, from complete failure of brain division to almost complete division. HPE is categorized by the degree of brain division into alobar, semilobar, lobar, and middle interhemispheric variants. Individuals may have only minor clinical signs without brain abnormalities, known as microform HPE. HPE is a multifactorial condition, in which environmental factors interact with genes to produce varying phenotypes. At least 12 HPE-associated genes have been identified. One such gene, *TGIF* (*TG-Interacting Factor*), is mapped to chromosome 18p11.3, and this gene product may affect members within the TGF- β family.

Previous research has suggested that genotype-phenotype correlations in nonsyndromic HPE are due to alterations of other HPE-associated genes. Therefore, the goal of our study is to determine whether such correlations exist in patients with *TGIF* alterations

Under our protocol, patients with HPE and available relatives are tested via sequencing for mutations in HPE-associated genes, including *TGIF*. A literature search for patients with alterations in *TGIF* and HPE was also performed.

A total of 30 patients with HPE and mutations in *TGIF* (or HPE-affected first-degree relatives of mutation-positive patients) were identified. From the literature, we found 31 patients with cytogenetic abnormalities affecting the *TGIF* locus detected by one chromosome analysis. Seventeen percent of patients had alobar HPE, 20% had semilobar HPE, 7% had lobar HPE, 40% had microform HPE, and 17% had no symptoms. Males and females are equally represented in this cohort.

Alterations in *TGIF* account for a small proportion of HPE. Preliminary analysis indicates that patients with *TGIF* alterations may be less severely affected, with a large percentage of patients displaying only microform HPE or no clinical signs.



A. KEATON



S. PASRICHA

11:15 A.M.

Quantitative trait loci analysis of nonalcoholic steatohepatitis

SARINA PASRICHA, Medical Fellow, Northwestern University, The Feinberg School of Medicine

Mentor: Richard M. Green, M.D., Northwestern University, The Feinberg School of Medicine

■ Nonalcoholic steatohepatitis (NASH) is one of the most common causes of liver disease in the United States. NASH is associated with the “metabolic syndrome,” which includes insulin resistance, obesity, and dyslipidemia. Recent evidence indicates that NASH is a polygenic disease with a strong genetic component for both susceptibility and progression of steatohepatitis. Unfortunately, the genetic factors responsible for the pathogenesis of NASH remain poorly understood. The purpose of this study was to identify the genetic loci associated with steatosis and hepatic inflammation using quantitative trait loci (QTL) analysis. QTL analysis is a well-established genetic technique to identify the genetic loci that are important for polygenic diseases. It has been applied to many polygenic diseases, yet not NASH.

Appropriate breeding between C57BL/6J (steatohepatitis-susceptible) and A/J (steatohepatitis-resistant) mice was conducted. A high-fat, high-calorie diet was fed for eight weeks to the F2 generation of these mice. QTL analysis is currently being used to identify the genetic loci associated with hepatic inflammation. Hepatic interleukin-1- β levels are being used as the quantitative trait, based on preliminary studies that have shown that hepatic interleukin-1- β protein and gene levels are highly expressed in C57BL/6J mice, but not in A/J mice.

Confirmation of the pathophysiologic importance of these QTLs and identification of the relevant genes are essential to enhance our understanding of the pathogenesis of NASH. This project utilizes a validated model of steatohepatitis, and state-of-the-art genetic and molecular biological techniques, to enhance our understanding of the pathogenesis and susceptibility to steatohepatitis. This increased understanding of the mechanisms responsible for steatohepatitis will allow for the design of rational therapies for this hepatic disease that affects millions of Americans.

11:30 A.M.

Structural shifts in heat shock RNA-1: a mechanism for thermosensing**PATRICK VARLEY**, Medical Fellow, New York University School of Medicine

Mentor: Evgeny Nudler, Ph.D., New York University School of Medicine

■ The cellular heat shock response is a highly conserved protective mechanism critical for the survival of cells under a variety of potentially harmful environmental conditions. Heat shock transcription factor 1 (HSF1) plays an important role in this pathway by inducing an array of cytoprotective proteins. Cellular stress causes trimerization of inactive HSF1 monomers, leading to activation of the heat shock pathway. Previously, we have shown that activation of HSF1 is mediated by a ribonucleoprotein complex consisting of translation elongation factor eEF1a and a novel noncoding RNA, heat shock RNA-1 (HSR1). RNA thermosensors have been described in other cellular contexts, and we hypothesize that temperature-mediated changes in the structure of HSR1 may facilitate interaction with eEF1a and subsequent activation of HSF1.

MFOLD analysis of the previously cloned and sequenced HSR1 predicted that significant structural shifts accompany temperature change. To investigate the validity of these predictions, we probed the structure of *in vitro* transcribed HSR1 by using nuclease digestion under single hit conditions at temperatures from 25 to 43°C. Maps of the nuclease digests were created through primer extension of the samples with ³²P-labeled primers, followed by separation of the resulting cDNA on polyacrylamide gel and visualization by autoradiography. To investigate the role that eEF1a may play in stabilizing conformational changes of HSR1, we first incubated the RNA with eEF1a at temperatures from 25 to 43°C and then subjected it to the same nuclease mapping techniques. By analyzing the digestion patterns from these experiments, we were able to develop crude maps of HSR1's temperature-dependent secondary structure.

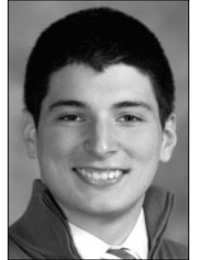
While initial data have allowed us to better understand the secondary structure of HSR1, the precise structural shifts that may cause activation of HSF1 as a result of temperature change remain unclear. Investigation is ongoing with regard to the critical role of HSR1 in the heat shock pathway.

11:45 A.M.

Whole-genome association study and fine mapping of a locus for malignant histiocytosis in the Bernese mountain dog**ABIGAIL SHEARIN**, Research Scholar, University of Pennsylvania School of Veterinary Medicine

Preceptor: Elaine Ostrander, Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Malignant histiocytosis (MH) belongs to a group of histiocytic disorders representing a broad range of clinical presentations, from the benign cutaneous histiocytoma to the severe malignant histiocytosis. Bernese mountain dogs (BMDs) exhibit a high prevalence of malignant histiocytosis: 20% of BMDs in the United States succumb to MH. This breed-specific prevalence strongly indicates a genetic predisposition. While MH is clearly genetic in the BMD, the mode of inheritance and the number of genes involved are unknown. Our goal is to determine a gene, and the mutation within that gene, involved in the increased prevalence of MH in the BMD. To find a region associated with MH, a whole-genome association study using Illumina SNPChip technology was performed. A total of 238 DNA samples was used: 115 from affected BMDs and 123 from control BMDs. A region of approximately 30 Mb was found with a high association with MH. An additional 230 SNPs have been genotyped using the high-throughput genotyping system SNPlex, increasing the density of SNPs and reducing the 30-Mb region to a manageable size for mutation detection. For SNPlex, 327 BMD samples were used: 175 from affected BMDs and 152 controls. Thus far, the 4 most highly associated SNPs, with *P* values $< 1 \times 10^{-6}$, fall within a 1-Mb region. When we include multiple immune cell cancers in our analysis, these associations increase, with *P* values $< 1 \times 10^{-7}$. Haplotype analysis is currently underway, and the smallest associated region is being sequenced with the goal of determining the gene and mutation responsible. The obvious implication of this study is the possibility of eliminating this devastating disease from the BMD. More importantly, however, we may expand the knowledge of the inheritance patterns of several immune cell cancers that have significant impacts on the lives of both humans and canines.



P. VARLEY



A. SHEARIN

TUESDAY
ATRIUM

POSTER C1

Examination of the effects of inhibiting TrkB signaling on limbic epileptogenesis in Kv1.1 null mutant mice

SIMA YAZDANI, Medical Fellow, Duke University School of Medicine

Mentor: James McNamara, M.D., Duke University



S. YAZDANI



H. K. BAHARANYI

■ Understanding partial epileptogenesis in cellular and molecular terms may provide targets for developing effective preventive therapies. A commonly studied animal model of epileptogenesis is kindling, in which pathological activity in the form of focal seizures induces progressively increased excitability culminating in severe epilepsy. Striking increases in expression of brain-derived neurotrophic factor (BDNF) were identified in the hippocampus during epileptogenesis. Increased phosphorylation of the BDNF receptor, TrkB, a surrogate measure of activation, was identified in the mossy fiber pathway of hippocampus in multiple models. This led to the hypothesis that TrkB activation is required for limbic epileptogenesis in the kindling model. It was demonstrated that a conditional deletion of TrkB eliminated epileptogenesis in the kindling model.

These findings raise a key question: does inhibition of TrkB signaling prevent limbic epileptogenesis in additional models or is it unique to the kindling model? To address this question, I will explore whether there is increased TrkB kinase activity in Kv1.1 null mutant mice by using biochemical and immunohistochemical techniques. The Kv1.1 null mutant mouse has been selected because loss-of-function mutations of this gene cause limbic epilepsy in humans and mice. If an increase in TrkB kinase activity is noted in these mice, the next question will focus on whether limiting TrkB kinase activity inhibits limbic epileptogenesis in Kv1.1 null mutant mice. To inhibit TrkB kinase activity, I will use a recently developed TrkB^{BF616A} mutant mouse. These experiments will provide information critical to understanding the potential value of TrkB as a molecular target for the development of a specific and effective antiepileptogenic.

POSTER C2

Tuberous sclerosis complex signaling regulates EphA-mediated axon guidance

HASANI K. BAHARANYI, Medical Fellow, Yale School of Medicine

Mentor: Mustafa Sahin, M.D., Ph.D., Children's Hospital Boston, Harvard Medical School

■ Tuberous sclerosis complex (TSC) is a disease characterized by the presence of hamartomas in the brain known as cortical tubers. Up to 90% of TSC patients have epilepsy, and it has been previously hypothesized that the cortical tubers cause seizures in TSC patients. However, mounting evidence shows that TSC patients can have seizures without tubers and that nontuber regions of the cortex can be epileptogenic. Neuronal miswiring, independent of cortical tubers, may contribute to the neurological symptoms seen in this disease, as abnormal neural circuitry can cause neurocognitive defects. The establishment of precise neuronal wiring requires interactions between extending axons and environmental guidance cues. Ephrins and their corresponding receptors, Ephs, have been identified as regulators of axon guidance in the central nervous system, including the optic chiasm, dorsal lateral geniculate nucleus (dLGN), and barrel cortex. Given these data, we hypothesize that the TSC1/TSC2 complex plays an important role in axon guidance by regulating the ephrin-EphA signaling pathway.

To investigate the role of the TSC1/TSC2 complex in axon guidance, we first demonstrated that key molecules in the TSC-Rheb-mTOR pathway are highly expressed in early postnatal purified retinal ganglion cell (RGC) axons. Using an anterograde tracer, we next showed that RGCs of Tsc2 ^{+/-} mice have abnormal axonal projections to the LGN. We then used a growth cone collapse assay to show that RGCs from these mice are less sensitive to the repulsive cues of ephrin-A1, *in vitro*. Lastly, we demonstrated that activation of Eph receptors leads to inactivation of the mTOR pathway.

Taken together, our data suggest that the TSC-Rheb-mTOR signaling pathway interacts with the ephrin-Eph receptor system to regulate axon guidance in retinogeniculate projections. These observations provide a potential mechanism for the neurological symptoms observed in TSC patients, and they identify a prospective target for therapeutic intervention.

POSTER C3

Glucose transport dysfunction in Alzheimer's disease

DAVID HENRY PERLMUTTER, Medical Fellow, University of Rochester School of Medicine and Dentistry

Mentor: Berislav V. Zlokovic, M.D., Ph.D., University of Rochester School of Medicine and Dentistry

■ While mutations in the production and processing of the amyloid precursor protein (APP) have been identified in select cases of early-onset familial Alzheimer's disease (AD), the vast majority of AD is sporadic and late onset, showing amyloid plaque accumulation in the brain parenchyma. Neurovascular dysfunction of amyloid-beta (A β) clearance—either due to low levels of low-density lipoprotein receptor-related protein-1, the primary receptor for A β clearance across the blood-brain barrier (BBB) from brain to blood, or due to increased levels of the receptor for advanced glycation end products (RAGE), the receptor for A β transport across the BBB from blood to brain—could cause the elevated A β and vascular lesions seen in AD.

In addition to abnormal neurovascular A β clearance, there is promising evidence that cerebral glucose metabolism is altered in AD. We believe that this may be due to neurovascular dysfunction in glucose transport at the BBB. AD patients show decreased glut-1 protein expression in brain capillaries and decreased surface area available for glucose transport, suggesting that the AD brain experiences a glucose shortage due to glut-1 deficiency at the BBB.

To study the effect of decreased glucose transport at the BBB on AD pathology, we have successfully developed a mouse model that is both APP overproducing and glut-1 heterozygous. When these mice age sufficiently, we plan to show increased A β plaque densities, decreased blood vessel size, decreased blood flow, increased neuronal loss and apoptosis, and decreased behavioral performance relative to their APP-overproducing controls.

Additionally, we are studying the link between RAGE function and glucose transport in AD mice (Swe+/-). Following two months of treatment with a RAGE antagonist, AD mice aged 11–13 months show increased glut-1 levels by immunohistochemical staining and decreased blood flow by 14-C iodoantipyrine uptake. We are currently finishing studies of glucose uptake and neurofilament densities in these mice.

POSTER C4

Synaptic adhesion-like molecule 5 (SALM5) expression pattern in hippocampal neurons

SHILA AZODI, Research Scholar, Texas Tech University Health Sciences Center School of Medicine

Preceptor: Robert J. Wenthold, Ph.D., National Institute on Deafness and Other Communication Disorders, National Institutes of Health

■ Synaptic cell adhesion molecules (CAMs) have various roles in the central nervous system, including synaptogenesis, neuronal development, synaptic plasticity, and structural maintenance. A novel family of five CAMs called synaptic adhesion-like molecules (SALMs) was identified and found to interact with NMDA receptors. SALMs play a role in neurite outgrowth and synapse formation. The goal of this investigation is to study the expression and function of SALM5.

SALMs have a characteristic structure of leucine-rich repeat regions, an immunoglobulin C2-like domain, a fibronectin type 3 domain, and a transmembrane region. The C-terminus of SALMs 1–3 contains a PDZ-binding domain, allowing interaction with postsynaptic density protein 95 (PSD-95). The C-terminus of SALM4 and -5 does not have this motif. Initially, it was thought that all SALMs have a synaptic localization. In transfected hippocampal neurons, SALM5 shows punctuate structures, although they do not colocalize with PSD-95, indicating that it does not have a synaptic localization. Overexpression of SALM5 generates a pattern of neurite association, suggesting that SALM5 may play a role in the early development of the nervous system. It has been shown that SALM5 forms trans homomeric interactions. One possibility is that the trans homomeric interaction between SALM5 proteins maintains the SALM5 peri- or extrasynaptic localization. We will test this idea by expressing a gene coding for the N-terminus of SALM5 in Sf9 insect cells to purify the truncated protein. Hippocampal neurons will be grown in culture with the protein, which should block the SALM5 trans interactions. We will look at the neurons to observe any disruption in SALM5 localization or neurite association.

Further studies in transfected hippocampal neurons will examine the pattern of SALM5 localization with other scaffolding proteins and adhesion molecules. These experiments will help determine the expression pattern and possible functions of SALM5.



D. H. PERLMUTTER



S. AZODI

POSTER C5

Loss of Parkin-induced mitophagy with disease-causing mutations

DEREK PAUL NARENDRA, Research Scholar, University of Michigan Medical School

Preceptor: Richard Youle, Ph.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ Loss-of-function mutations in Park2, the gene coding for the E3 ubiquitin ligase Parkin, are a significant cause of early-onset Parkinson's disease. Disease-causing mutations are distributed throughout Parkin's various domains, including the N-terminal ubiquitin-like domain, the linker region, and the C-terminal RING-between-RING domain composed of a characteristic RING domain (RING1) and two atypical RING domains (IBR and RING2). Although the role of Parkin in neuron maintenance is unknown, recent work has linked Parkin to the regulation of mitochondria. Its loss is associated with swollen mitochondria and muscle degeneration in *Drosophila*, as well as mitochondrial dysfunction and increased susceptibility to mitochondrial toxins in other species. We have found that Parkin is selectively recruited to dysfunctional mitochondria with low membrane potential in mammalian cells. Following recruitment, Parkin mediates the engulfment of mitochondria by autophagosomes and the selective elimination of impaired mitochondria. Disease-causing mutations suggest a role for the ubiquitin-like domain in Parkin's recruitment to mitochondria and suggest that the RING1, IBR, and RING2 domains are necessary for Parkin recruitment and mitophagy. Additionally, these data suggest that loss of Parkin-induced mitophagy resulting from these missense mutations may underlie the development of early-onset Parkinson's disease in patients carrying Park2 mutations.



D. P. NARENDRA



R. PARKER

POSTER C6

Novel interaction of hereditary spastic paraplegia protein spartin (SPG20) with the endosomal sorting complex required for transport (ESCRT) protein HIST1

RELL PARKER, Research Scholar, University of California, Davis, School of Veterinary Medicine

Preceptor: Craig Blackstone, M.D., Ph.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ The hereditary spastic paraplegias (HSPs) are a group of inherited disorders that are characterized by spasticity and weakness in the legs of patients, though there may be additional clinical manifestations associated with this disease in some cases. From over 40 different known genetic loci, almost 20 gene products have been identified. One of the proteins that is currently under investigation is called spartin (SPG20), which was first identified in an Old Order Amish family and causes an autosomal recessive form of HSP that also is accompanied by shortness of stature, mental retardation, and dysarthria. Another HSP protein known as spastin (SPG4) is mutated in an autosomal dominant form of the disease. Spastin has been shown to bind CHMP1B, an endosomal sorting complex required for transport (ESCRT)-III protein. Spastin binds CHMP1B through its MIT (present in microtubule-interacting and transport proteins) domain, but although spartin shares a very similar MIT domain, it does not bind CHMP1B. Recently, a new protein, HIST1, which is also an ESCRT-III protein, was shown to bind spastin. Interestingly, we have evidence that it is also able to bind spartin. Using a single-point mutation, which we derived from the published crystal structure of the CHMP1B-spastin complex, we were able to disrupt HIST1 binding to both spastin and spartin in a yeast two-hybrid system. We are pursuing this interaction in mammalian cells and are interested to see whether the disruption of binding between spartin and HIST1 has any effect on the role that spartin has been shown to play in EGFR trafficking. Spartin and HIST1 are also both recruited to the midbody during cytokinesis, so we are investigating whether a disruption of this interaction will interfere with cytokinesis.

POSTER C7

Functional evidence of neuroprotection through administration of an engineered anti-apoptotic fusion protein following acute spinal cord injury

JAYESH P. THAWANI, Research Scholar, University of Michigan Medical School

Preceptor: Richard J. Youle, Ph.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ Traumatic spinal cord injury (SCI) affects approximately 10,000–20,000 people per year in the United States and contributes to significant functional and medical impairments. The psychosocial and financial burdens associated with SCI are shared by these unfortunate individuals and society at large.

Neuronal cell death and apoptosis are processes that occur at focal points during SCI. Factors known to have anti-apoptotic effects, such as Bcl-XL (a member of the Bcl-2 subclass of apoptotic mediators), are downregulated during this process. Bcl-XL has been shown to prevent neuronal apoptosis during development as well as in models of neurodegenerative disease and trauma. Fusion proteins (FPs) incorporating a nontoxic derivative of anthrax toxin have been shown to be effective in delivering compounds into the cytosolic compartment of mammalian cells. By engineering a Bcl-XL FP possessing this nontoxic derivative, we were able to target the intracellular delivery of Bcl-XL. In addition to preventing apoptosis as demonstrated in vitro, administration of Bcl-XL FP has been shown to increase the survival of dorsal and ventral spinal cord motor neurons following induced SCI in the rat. To further test the anti-apoptotic effects of Bcl-XL, we demonstrate its functional neuroprotective effects through a rat SCI model.

Animals treated with Bcl-XL FP five minutes following SCI induced at T13 exhibited statistically significant functional improvements compared to controls through improvements noted in hind-limb locomotor function (open field locomotor scores: 11.2 ± 0.6 vs. 6.3 ± 0.5 ; $P < 0.05$). An increase in ventral motor neuron survival, reductions in the loss of oligodendroglial-associated tissue (reflected as an increase in white matter spared), and decreases in gliotic scarring following SCI were observed in treatment groups using histological and immunohistochemical techniques. This study demonstrates that Bcl-XL FP can be delivered locally in an animal model of acute SCI to successfully reduce functional impairment, likely through its anti-apoptotic effects.

POSTER C8

Genome-wide association study for intracranial aneurysm

NIKHIL R. NAYAK, Medical Fellow, Yale School of Medicine

Mentor: Murat Gunel, M.D., Yale School of Medicine

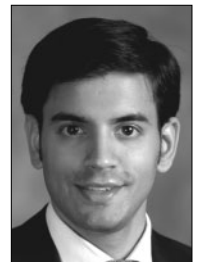
■ The rupture of intracranial aneurysm (IA) causes a form of hemorrhagic stroke with extremely high morbidity and mortality. While IA is a common disease, affecting up to 5% of the population, only a fraction of IA patients will develop subarachnoid hemorrhage (SAH) secondary to rupture. SAH carries the highest mortality of all types of stroke, with nearly 50% of individuals not surviving one month after the initial event. Despite clinical advances in the treatment of IA, the poor prognosis of SAH highlights the importance of early detection and treatment. While there are several known environmental risk factors associated with IA, a genetic contribution to the disease has long been suspected.

We hypothesize that both rare and common variants in the human genome play a role in predisposing an individual to the formation and rupture of IA. To study the common variants, our group conducted a multistage genome-wide association study of Finnish, Dutch, and Japanese cohorts, including more than 2,100 IA cases and 8,000 controls, using Illumina370Duo platform. The discovery phase in the European cohorts and subsequent replication study in the Japanese cohort identified loci on chromosomes 2q, 8q, and 9p with significant association with IA. These regions carried odds ratios of 1.24–1.36 and collectively account for 38–46% of the population-attributable risk of developing IA.

The loci on 2q and 8q are novel, whereas the region on 9p has been previously associated with multiple arterial diseases, including IA. Among the three regions, SOX17 on 8q is of particular interest. SOX17 has a key role in fetal and neonatal hematopoietic stem cells and is expressed in adult endothelial cells. Functional studies on SOX17 are currently underway.



J. P. THAWANI



N. R. NAYAK

POSTER C9

Prestin upregulation increases outer hair cell electromotility and is associated with cell death

CHRISTOPHER CHENG-YU LIU, Medical Fellow, Baylor College of Medicine

Mentors: John S. Oghalai, M.D., and William E. Brownell, Ph.D., Baylor College of Medicine

■ Outer hair cells (OHCs) improve hearing sensitivity by producing force that amplifies sound vibrations within the cochlea. This phenomenon, termed electromotility, is mediated by prestin, an OHC-specific membrane motor protein. Our laboratory recently created the *Tecta C1509G* mouse strain, which is more susceptible to noise-induced hearing loss and expresses 25% more prestin in its OHCs. We hypothesized that increased electromotility from prestin overexpression predisposes to OHC death.

We uniformly stimulated OHCs within a freshly excised mouse cochlear preparation by applying an external electric field using a sine wave current (1–40 kHz, 10 mA p-p). A laser doppler vibrometer was used to measure the electromotile responses. The electrically evoked displacements ranged between 0.5 and 3 nm, equivalent to that of moderate noise levels (30–60 dB SPL). *Tecta* heterozygote and homozygote OHCs produced larger displacements than wild-type OHCs by 4.58 ± 0.24 dB and 4.95 ± 0.15 dB, respectively (mean \pm SEM, $n=33-34$, $P<0.05$). We also controlled for movements not requiring prestin by testing *Prestin* knockout mice ($n=10$). As expected, no movements were detected.

After the stimulus, we stained each cochlea with propidium iodide, a vital dye that enters cells with compromised membranes. The proportion of stained OHCs was increased in *Tecta* heterozygote and homozygote cochleae ($42.3 \pm 5.5\%$ and $26.5 \pm 3.8\%$, $n=11$, $P<0.01$) compared to wild-type or *Prestin* knockout cochleae ($10.6 \pm 2.7\%$, $n=12$; $6.4 \pm 3.9\%$, $n=5$). Control cochleae labeled without stimulus application did not show these genotypic differences ($P=0.41$).

Our data demonstrate that OHCs with elevated prestin density have increased electromotile responses and higher rates of cell death after stimulation. This suggests that disorders of prestin regulation may underlie the pathogenesis of noise-induced hearing loss.



C. C.-Y. LIU



J. CHANG

POSTER C10

Molecular and functional changes associated with normal aging in the retina

JESSICA CHANG, Research Scholar, Duke University School of Medicine

Preceptor: Anand Swaroop, Ph.D., National Eye Institute, National Institutes of Health

■ Advanced age is a major risk factor for prevalent blinding diseases like diabetic retinopathy and age-related macular degeneration, yet little is known about the normal aging of the retina. Rod photoreceptors have been shown to be particularly vulnerable to the effects of aging. This study aims to elucidate changes in gene expression and metabolic function associated with normal aging of retinal neurons, particularly rod photoreceptors.

Flow cytometry was used to purify GFP (green fluorescent protein)-tagged rod photoreceptors from mouse retinas at ages ranging from 1.5 to 12 months, and mRNA was extracted to create gene expression profiles using Affymetrix chips. To complement these studies, mitochondrial respiratory function was compared using dissociated retinal cells from mice of different ages ranging from 1.5 to 18 months. Electroretinograms (ERGs) were also performed to measure functional changes in vision with age.

Candidate genes identified by microarray are being validated by quantitative polymerase chain reaction and immunohistochemistry to confirm expression level changes. Although mitochondria are widely implicated in many theories of aging, mitochondrial respiratory function showed little change across the spectrum of ages tested. ERGs, however, showed decreases in scotopic a and b waves, and in photopic b waves with increasing age.

Aging is a complex process that varies between cell types within the same individual, and evaluating gene profile changes in a single postmitotic cell type has great potential for identifying pathways in the aging process. The candidate genes found here provide a foundation for future experiments to explore potential pathways of normal aging and related functional adaptation, as well as a baseline for comparison with diseased aging.

POSTER C11

Real-time analysis of EEG in the elucidation of volition

LOGAN SCHNEIDER, Research Scholar, University of Florida College of Medicine

Preceptor: Mark Hallett, M.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ Cortical activity preceding movement intention is well documented. Recent studies have found that the awareness of the intent to move has a more complex relationship to brain activity, with the intention being progressively more perceptible. Additionally, the study of patients with Tourette syndrome and psychogenic movement disorders highlights the discrepancy between the sense of volition and the brain's movement-initiating activity. Subjectivity has limited the study of this phenomenon; however, better volition-brain activity discrimination lies at the core of the study of many movement disorders. Our aim is to predict the movements of healthy subjects in order to assess their movement intention and response to preemptive predictions.

Using 29-channel EEG and EMG recorded during spontaneously generated, isolated right wrist extension with no external cues, data were gathered on 120 movements over two calibration sessions and processed to predict movement prior to its occurrence. A threshold was researcher chosen, after EEG power spectrum analysis, to ensure >50% true positives and >10% false positives in the predictions made by the model. A light was turned on in response to EMG activity as well as at the time of prediction. Subjects reported their thoughts (intention or not) at the time of prediction.

Movement was verified with online EMG analysis.

It is possible with EEG analysis to predict movement prior to its occurrence. In such situations, subjects are aware only some of the time that they are intending to move. This demonstrates objectively that movement is initiated subconsciously. When the "effect of the movement" occurred prior to movement, this produced a sense of frustration, likely due in part to a distorted sense of agency.

POSTER C12

HermesC: low-power wireless neural recording system for freely moving primates

PAUL NUYUJUKIAN, Medical Fellow, Stanford University School of Medicine

Mentor: Krishna Shenoy, Ph.D., Stanford University

■ Neural prosthetic systems have the potential to restore lost functionality to amputees or patients suffering from neurological injury or disease. Current systems have primarily been designed for immobile patients, such as tetraplegics functioning in a rather static, carefully tailored environment. However, an active patient such as an amputee in a normal dynamic, everyday environment may be quite different in terms of the neural control of movement. To study motor control in a more unconstrained natural setting, we seek to develop an animal model of freely moving humans. Therefore, we have developed and tested HermesC-INI3, a system for recording and wirelessly transmitting neural data from electrode arrays implanted in freely moving rhesus macaques. This system is based on the integrated neural interface (INI3) microchip, which amplifies, digitizes, and transmits neural data across a ~900-MHz wireless channel. The wireless transmission has a range of ~4 m in free space. All together, this device consumes 15.8 mA and 63.2 mW. On a single 2 A-hour battery pack, the device runs contiguously for approximately six days. The smaller size and power consumption of the custom IC allows for a smaller package (51×38×38 mm³) than previous primate systems. The HermesC-INI3 system was used to record and telemeter one channel of broadband neural data at 15.7 kSps from a monkey performing routine daily activities in the home cage.



L. SCHNEIDER



P. NUYUJUKIAN

TUESDAY
 ATRIUM

POSTER C13

Behavioral and neural correlates of decision making after sleep deprivation: an fMRI study

MICHELLE BINDER JONELIS, Medical Fellow, University of California, San Francisco, School of Medicine

Mentor: Sean P.A. Drummond, Ph.D., University of California, San Diego



M. B. JONELIS

■ Sleep deprivation (SD) is highly prevalent, and many individuals are required to routinely work without adequate sleep. Previous research shows SD can impair cognition and brain function across a variety of domains. We examined how SD affects brain systems underlying three specific components of decision making: 1) risk preference, 2) information integration, and 3) probability weights. Fifteen subjects performed three decision-making tasks during fMRI while “well rested” (six nights of 9 hours in bed/night) and after SD (either 24 hours of no sleep or five nights of 4 hours in bed/night).

Behaviorally, we found SD to produce 1) altered risk preference, with SD subjects becoming more risk seeking when trying to win money and more

risk averse when trying to not lose money, for the highest risk decisions; 2) less integration of multiple pieces of information into a decision; and 3) no change in probability weights associated with uncertain outcomes. Regarding brain activation, we found SD to produce 1) decreased activation in right insula, right nucleus accumbens, and left cingulate body while making high-risk decisions in an attempt to win money; 2) decreased activation in the bilateral dorsolateral prefrontal cortices and increased activation in the bilateral ventrolateral prefrontal cortices during information integration; and 3) increased activation in the left insula and left inferior parietal regions when choosing a gamble with known probabilities over a sure win.

Taken together, these results suggest sleep-deprived individuals may show impairment in their ability to evaluate risky decisions. Subjects showed blunted emotional responses to high-risk decisions, a diminished capacity to use all the provided information to make decisions, and greater anticipation of gambles paying off. In applied settings, this may mean sleep-deprived individuals make riskier choices while not engaging the typical cognitive or affective considerations used when well rested.

POSTER D1

Amplifying the Wnt pathway to enhance bone regeneration

STEVE MINEAR, Medical Fellow, Stanford University School of Medicine

Mentors: Jill Helms, D.D.S., Ph.D., Stanford University School of Medicine, and Roel Nusse, Ph.D., Howard Hughes Medical Institute, Stanford University School of Medicine

■ Skeletal tissue retains the remarkable ability to regenerate lost or damaged tissue into adulthood. Wnt signaling is one of the central regulators of osteogenesis during this repair process, mediating osteoblastic differentiation, expansion, and activity. After skeletal injury, Wnt signaling is locally and transiently upregulated, and analyses of mutations affecting the Wnt pathway have established an important role for Wnt during the subsequent regenerative program. The dosage and duration of the Wnt signaling here is critical: repressing Wnt signaling inhibits bone regeneration; likewise, constitutively active Wnt signaling also inhibits bone regeneration.

In this project, we show that deletion of *Axin2*, an intracellular, negative regulator of Wnt signaling, creates an amplified Wnt response following injury. This enhanced Wnt signaling induces a more robust osteogenic response in the injury site. Osteoprogenitors proliferate and differentiate more robustly in *Axin2*^{-/-} injury sites and subsequently produce a larger regenerate. This suggests a novel mechanism for improving current therapies designed to increase or retain bone mass, because many or all FDA-approved therapies focus not on increasing osteoblastic functions, but in repressing osteoclastic functions.

Based on these data, we developed a biomimetic approach to transiently activate Wnt signaling during bone repair. By packaging Wnt3a into liposomes, we were able to prevent their in vivo degradation, which has impeded previous efforts to use Wnt proteins as a viable therapy. Our liposomal application resulted in the rapid healing of skeletal defects. The regenerative response matched that of *Axin2*^{-/-} mice: both proliferation and differentiation of new osteoblasts were increased, resulting in a larger amount of new bone matrix. This clinically relevant application of liposomal Wnt3a is of broad interest to the medical community, and it offers new ways to treat skeletal injuries that have proven refractive to other therapies.

POSTER D2

The mechanism and physiologic significance of osteoprotegerin repression by nuclear factor of activated T cells c1 during osteoclastogenesis

ROSALYN M. SULYANTO, Medical Fellow, Harvard School of Dental Medicine

Mentors: Laurie H. Glimcher, M.D., Harvard School of Public Health and Brigham and Women's Hospital, and Antonios O. Aliprantis, M.D., Ph.D., Brigham and Women's Hospital

■ Bone homeostasis requires a balance between bone-forming osteoblasts (OBs) and bone-resorbing osteoclasts (OCs). OC formation requires the OB-derived cytokine receptor activator of nuclear factor κ B ligand (RANKL). The transcription factor nuclear factor of activated T cells c1 (NFATc1) is a master regulator of OC differentiation downstream of RANKL. Recently, our laboratory confirmed the role of NFATc1 in osteoclastogenesis in vivo using a conditional knockout strategy. Gene profiling of NFATc1-deficient (NFATc1 $\Delta\Delta$) OC precursors (OcPs) stimulated with RANKL surprisingly revealed high levels of expression of osteoprotegerin (OPG), a soluble decoy receptor for RANKL that negatively regulates OC differentiation, previously thought to be derived from OBs in bone. This observation provides the first evidence that OcPs are capable of producing OPG when stimulated with RANKL and implies a novel negative control mechanism for OC differentiation. Cell culture experiments were performed to elucidate the mechanism of NFATc1-mediated repression of OPG during osteoclastogenesis.

In vitro, RANKL induced OPG mRNA and protein expression in NFATc1 $\Delta\Delta$, but not wild-type, OcPs. In contrast, treatment of wild-type OcPs with RANKL in the presence of cyclosporine A, an inhibitor of the NFAT activating phosphatase calcineurin, leads to OPG expression. Infection of NFATc1 $\Delta\Delta$ OcPs with a constitutively active form of NFATc1 (caNFATc1) decreased RANKL-mediated OPG expression. A chromatin immunoprecipitation assay determined that after 3 days of RANKL treatment, NFATc1 was recruited to the OPG promoter. Lastly, in 293 cells, caNFATc1 repressed the basal activity of a reporter plasmid containing the proximal 3.6 kb of the OPG promoter upstream of luciferase.

Taken together, these results uncover a previously unrecognized regulatory loop downstream of RANKL: calcineurin/NFATc1-mediated repression of OPG expression. Currently, bone marrow transfer experiments using OPG-deficient hematopoietic stem cells are being performed to determine the physiologic relevance of OcP-derived OPG in vivo.



S. MINEAR



R. M. SULYANTO

POSTER D3

Characterization and expansion of human spermatogonial stem cells for the derivation of pluripotent cell lines

RAUL I. CLAVIJO, Medical Fellow, University of California, San Francisco, School of Medicine

Mentors: Renee A. Reijo Pera, Ph.D., Stanford University School of Medicine, and Paul J. Turek, M.D., The Turek Clinic

■ In the past few years, embryonic stem cell (ESC)-like cells have been derived from spermatogonial stem cells (SSCs) isolated from mouse and, more recently, human testicles. A protocol for the derivation of these ESC-like cells has been well established in the mouse. This has been facilitated by the availability of a system for the expansion of mouse SSCs. In humans, no system for SSC propagation exists. The importance of developing such a system is highlighted by the fact that SSCs make up about 0.03% of the testicular germ cell population. Furthermore, researchers are often limited to small amounts of human testicular tissue from biopsies. To address this issue, we sought to develop a protocol to isolate and propagate human spermatogonia. We also aimed to characterize the expression of pluripotency and germ cell markers in human SSCs to facilitate their enrichment.

We have found that human spermatogonia can be enriched significantly by differential plating using gelatin and laminin/matrigel-coated tissue culture dishes. Human spermatogonia then survived in vitro for up to two weeks. However, minimal mitotic activity is seen, with most spermatogonia differentiating into spermatid-like cells or undergoing apoptosis. All human spermatogonia maintained in vitro express the germ cell marker VASA, with a subpopulation expressing the stem cell markers OCT-4, PLZF, and C-KIT. Acrosin expression can be detected in spermatid-like cells that appear after about two weeks of spermatogonial culture. To further study human spermatogonia and their potential to become pluripotent cells, we have transduced them with SV40 large T antigen and TERT (telomerase reverse transcriptase) by using a lentiviral vector in an effort to immortalize them.

Overall, our studies have identified a putative stem cell population among cultured human spermatogonia that we will continue to characterize. We have also taken the first steps to derive an immortalized human spermatogonial line for future studies.



R. I. CLAVIJO



M. J. TOKITA

POSTER D4

Conformational state of heat shock protein 90 governs binding of structurally diverse small-molecule inhibitors

MARI JOHANNA TOKITA, Research Scholar, The Warren Alpert Medical School of Brown University

Preceptor: Len Neckers, Ph.D., National Cancer Institute, National Institutes of Health

■ Heat shock protein 90 (Hsp90) is a molecular chaperone that facilitates the conformational maturation and function of a diverse group of protein clientele, including mutated or aberrant proteins that abet cancer cell growth and survival. Recognition of Hsp90's role in supporting these oncogenic signaling proteins has buoyed interest in the utility of Hsp90 inhibitors as anticancer drugs.

Hsp90 undergoes a conformational cycle driven by ATP binding and hydrolysis and modulated by co-chaperones to convert its client proteins to their stabilized, activated forms. Hsp90 protomers are constitutively dimerized via their C-terminal domains. ATP binding induces transient N-terminal dimerization, converting Hsp90 from an "open" to a "closed" conformation. Upon ATP hydrolysis, the N-domains separate and the client protein is released, completing the chaperone cycle. Two structurally distinct Hsp90 inhibitors, geldanamycin (GA) and a purine scaffold inhibitor (PU), block Hsp90 chaperone function by interfering with this ATPase cycle. We endeavored to ascertain the conformational status of Hsp90 bound to each of these compounds as a first step in developing strategies to enhance cancer cell sensitivity to these drugs.

Our preliminary results suggest that GA and PU bind different populations of Hsp90. We performed sequential pulldown studies using drug-conjugated beads to show that the pool of Hsp90 bound by GA is contained within a less selective pool of Hsp90 that binds to PU. We also determined that Hsp90 bound to PU co-precipitates with clients and co-chaperones, while GA-bound Hsp90 does not. These findings suggest that PU binds Hsp90 in both an open and closed configuration, whereas GA binds primarily to open Hsp90. We will further investigate this hypothesis by examining the effect of client and co-chaperone overexpression on drug binding, by determining the affinity of GA and PU for constitutively open or closed Hsp90 mutants, and by pursuing structural studies of purified GA- and PU-bound Hsp90.

POSTER D5

Role of the Pink1-Parkin in lipid-induced autophagy in skeletal muscle and liver cells

SARAH ELIZABETH RUSK, Research Scholar, Case Western Reserve University School of Medicine

Preceptor: Michael N. Sack, M.D., Ph.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ Eukaryotic cells utilize many mechanisms to promote stress-survival. Autophagy is a major degradation system to facilitate cellular homeostasis via recycling damaged or unnecessary intracellular components. Mitochondrial autophagy (mitophagy) enables basal turnover of the mitochondria population and removal of dysfunctional mitochondria. As defective mitochondria are proapoptotic proteins and generate reactive oxygen species, their cellular elimination enhances cellular survival. Accordingly, autophagy is induced by cellular stress, including starvation and free fatty acid insult. Impaired autophagy is implicated in the pathogenesis of diabetes as mice with autophagy-deficient β cells develop glucose intolerance following high-fat feeding. Interestingly, the Pink1-Parkin pathway, which modulates mitochondrial integrity and function, has recently been implicated in the regulation of mitophagy. Furthermore, polymorphisms in Pink1 and Parkin genes associate with increased risk of type 2 diabetes. We hypothesize that the Pink1-Parkin program modulates the response to the diabetogenic stress of lipid accumulation via regulating mitophagy.

As skeletal muscle and the liver are central in the development of type 2 diabetes, we established a model system to examine autophagy in skeletal muscle and liver cells in response to fat overload. Steatosis is induced in C2C12 myoblasts and HepG2 cells following incubation with the unsaturated fatty acid oleate. In both cell lines, steatosis is associated with the activation of autophagy. We will now genetically manipulate the Pink1-Parkin program in these cells to evaluate their effects on steatosis and autophagy. Further characterization of this regulatory program may delineate mechanisms whereby Pink1 and Parkin modulate autophagy and steatosis and may advance our understanding of these proteins in the pathogenesis of diabetes.

POSTER D6

Parathyroid hormone-related protein overexpression protects chondrocytes subjected to injurious cyclic tensile strain

DEAN WANG, Research Scholar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Preceptor: Rocky Tuan, Ph.D., National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health

■ Hyaline cartilage, the load-bearing tissue of articulating joints, has very limited repair and regeneration capacities. In osteoarthritis, mechanical and inflammatory stresses cause chondrocytes to undergo a catabolic response and apoptosis, ultimately leading to matrix degradation. Current engineered cartilage constructs have failed to provide sufficient mechanical function in vivo partly due to these stressors already present in the joint. For engineered cartilage to meet its functional demands, either the underlying arthritis must be cured or the engineered cartilage must be made resistant to the disease.

Parathyroid hormone-related protein (PTHrP) is a key mediator in suppressing hypertrophic differentiation of chondrocytes during endochondral ossification and increases the survival of chondrocytes under stressed conditions by inhibiting apoptosis. Interestingly, the cellular changes that occur during hypertrophic differentiation resemble the changes encountered in an arthritic phenotype, including increased matrix metalloproteinase (MMP) production, increased apoptosis, and decreased matrix synthesis. Based on these similarities, our central hypothesis is that PTHrP gene therapy will prevent cells subjected to mechanical injury from exhibiting an arthritic phenotype, thus decreasing chondrocyte catabolism and apoptosis.

Primary bovine chondrocytes were transfected with plasmids constitutively expressing two PTHrP isoforms, 1-173 and 1-141. Cells were cultured onto BioFlex plates and subjected to injurious 16% cyclic tensile strain. Gene transcripts representative of anabolic/chondrogenic differentiation, matrix remodeling, hypertrophy/injury, and apoptosis were analyzed using real-time RT-PCR. Both PTHrP isoforms significantly suppressed loading upregulation of hypertrophic markers, including collagen type X and alkaline phosphatase, during 48 hours of continuous cyclic strain. PTHrP-treated cells produced less nitric oxide and had increased cell viabilities compared to control cells after strain-induced injury. In conclusion, gene-based modification of chondrocytes using PTHrP is a promising method for protecting regenerated cartilage from the damaging stressors of osteoarthritis.



S. E. RUSK



D. WANG

TUESDAY
ATRIUM

POSTER D7

Metabolic stress of diabetes induces autophagy in the heart

KARI A. WELLNITZ, Medical Fellow, University of Texas Health Science Center at Houston

Mentor: Heinrich Taegtmeier, M.D., D. Phil., University of Texas Health Science Center at Houston

■ The heart in diabetes is characterized by dysregulated glucose metabolism, intracellular lipid accumulation, and increased generation of reactive oxygen species, all of which can contribute to the development of heart failure. Autophagy is an important pathway for the degradation of organelles and long-lived proteins via the lysosome. This process has been shown to promote cell survival by eliminating oxidized proteins and damaged mitochondria. We propose that autophagy is increased in the hearts of type 2 diabetics and plays a role in modulating diabetes-induced cellular damage.

To test this hypothesis, we used two murine models of type 2 diabetes, NONcNZO10/LtJ (RCS10) mice and *db/db* mice. In *db/db* mice, the diabetic phenotype leads to decreased cardiac performance. In contrast, the hearts of diabetic RCS10 mice exhibit generalized hypertrophy but are not functionally impaired. Although patients with type 2 diabetes develop congestive heart failure at a rate 2.5 times that of nondiabetics, not all diabetic patients develop cardiac dysfunction. We used these two models to elucidate the role of autophagy in adaptation and maladaptation of the heart to diabetes. The protein levels of specific autophagosome marker LC3-II and mediators of autophagy Beclin1 and Atg5-12 conjugate were significantly increased in 15-week-old RCS10 mice who had been hyperglycemic (nonfasting blood glucose >250 mg/dl) for at least 3 weeks. Hearts from age-matched *db/db* mice had significantly increased protein levels of LC3-II, Beclin1, Atg5-12 conjugate, and Atg10. Our preliminary results suggest that autophagy is increased in the diabetic heart regardless of the underlying functional status of the myocardium. We are currently quantifying transcript levels of autophagy-related genes, evaluating flux through the autophagic pathway, and examining the molecular mechanisms driving increased autophagy. These experiments will provide novel insights into the role of autophagy in the diabetic heart.



K.A. WELLNITZ



M. HODAVANCE

POSTER D8

Improved biocompatibility of implanted devices through seeding with adult stem cells

MICHAEL HODAVANCE, Medical Fellow, Duke University School of Medicine

Mentors: Bruce Klitzman, Ph.D., and W. Monty Reichert, Ph.D., Duke University School of Medicine

■ Implanted devices often fail because of the foreign body response involving inflammation, biofouling, and formation of a relatively avascular fibrous capsule around the device. This reaction is seen with glucose biosensors, as well as other chronically implanted devices, and is a limiting factor for their use. Understanding and improving this reaction could therefore increase the applicability of various implanted devices. The foreign body reaction in adipose tissue is less aggressive than that seen in lean tissue. Recent suggestions are that subcutaneous implants coated with adipose-derived stem cells (ASCs) induce a less robust foreign body response and have a different cytokine release profile compared to fibroblast-coated implants. However, the mechanisms by which this occurs are still poorly understood. Our goal was to study ASC-coated implants using a window chamber model in which the tissue environment *in vivo* can be directly and noninvasively assessed in real time.

ASCs were isolated and seeded onto nonfunctional glucose sensors using a previously optimized protocol. Untreated and ASC-coated sensors were implanted into the dorsum of rats, and the overlying skin was excised. Then, a transparent, 1-cm-diameter window was implanted overlying the sensor, allowing for real-time direct visualization and quantification of the microvascular architecture adjacent to the sensor. Laser Doppler flowmetry was also used to quantify blood flow beneath the window. Sodium fluorescein was injected (10 mg/kg) to enhance the contrast between the microvessels and the adjacent tissue. Following implantation, data were acquired on day 0, 4, 7, 11, and 14, and up to 28 days if biofouling or sensor migration prevented continued acquisition. Since sodium fluorescein diffuses easily through tissue and the emitted wavelength is absorbed strongly by hemoglobin, excellent contrast was achieved within 10 minutes of injection. This technique enables us to do mechanistic studies of microvascular network remodeling in response to implanted devices.

POSTER D9

Analyzing variation in gene regulation in humans: global analysis of NF- κ B binding using ChIP-Seq in different individuals

MAYA KASOWSKI, Medical Fellow, Yale School of Medicine

Mentor: Michael Snyder, Ph.D., Yale School of Medicine

■ Humans have diverse phenotypes, and it remains unclear how much diversity is due to differences in gene content (e.g., sequence differences) and how much is due to differences in gene regulation. We sought to examine the variation in transcription factor binding upon different humans using chromatin immunoprecipitation followed by DNA sequencing (ChIP-Seq). I have mapped the binding sites of a key regulator of the immune system, NF- κ B, as well as Pol II in nine HapMap lymphoblastoid cell lines of African, European, and Asian ancestry. Divergence in NF- κ B binding may help explain differences in the response to pathogens, inflammatory processes, and the progression of certain cancers. We have also measured gene expression in each cell line following TNF- α stimulation with Affymetrix GeneChips in order to correlate differences in binding profiles with expression.

I have completed mapping Pol II and NF- κ B binding sites in all nine individuals. To cover all possible targets, we generated approximately 12 million mapped reads from three biological replicates on Illumina sequencers. In the case of Pol II, inspection of the signal tracks suggests that most targets are the same, but some differences have been observed. More variation is observed in NF- κ B binding sites among individuals. We are currently conducting a statistical analysis to quantify these differences in binding sites and link them to copy number variations and single nucleotide polymorphisms.

This study is expected to shed insight into how much variation exists between individuals at the level of transcription factor binding. We speculate that this may be a major form of variation in humans.

POSTER D10

Biomechanical control of microRNA expression and vascular homeostasis

GUADALUPE VILLARREAL JR., Medical Fellow, Harvard Medical School

Mentor: Guillermo Garcia-Cardena, Ph.D., Harvard Medical School

■ The transcription factor Kruppel-like factor 2 (KLF2) serves as a critical integrator of multiple endothelial functions, conferring antithrombotic, anti-inflammatory, and anti-adhesive properties to the vascular endothelium. The suggested importance of this transcription factor is highlighted by studies showing that its expression is selectively increased under “atheroprotective” flow conditions simulating those found in regions of the carotid artery resistant to atherosclerosis.

MicroRNAs are small, noncoding RNAs that function by controlling the posttranscriptional expression of genes. The influence of biomechanical stimuli on endothelial microRNA expression and the impact that microRNAs have in vascular homeostasis remain largely unknown. To this end, we investigated the biomechanical dependency of microRNA expression in endothelial cells and the role of microRNAs in the regulation of KLF2. A real-time PCR screen of 667 human microRNAs revealed the distinct regulation of microRNAs by atheroprotective flow compared to static (no flow) conditions. Knock down of Dicer1 by siRNA resulted in a twofold increase in KLF2 mRNA expression under static conditions. Additionally, when a luciferase expression vector containing the 503 base pair human KLF2 3'UTR was transfected into endothelial cells, a 54% and 72% decrease in expression, as compared to a luciferase control, was observed under static and atheroprotective flow conditions, respectively. In order to identify regions critical for the regulation of the KLF2 3'UTR, we created four luciferase reporter constructs containing a series of fragments of the KLF2 3'UTR. Using this approach, we located the region of interest to two nonoverlapping fragments, and have divided them into subfragments for further luciferase expression analysis. Candidate microRNAs contained within the subfragments of interest will be compared against the list of differentially regulated microRNAs identified by the real-time PCR screen.

Collectively, the findings derived from our studies should lend further insights into the complex microRNA regulatory mechanisms governing endothelial function and vascular homeostasis.



M. KASOWSKI



G. VILLARREAL JR.

POSTER D11

Testing of a novel metabolite/transcript network for regulation of gluconeogenesis

DIVAKAR GUPTA, Medical Fellow, Duke University School of Medicine

Mentor: Christopher B. Newgard, Ph.D., Duke University School of Medicine



D. GUPTA

Metabolites represent the end product of cellular reactions and are capable of influencing gene expression, making them ideal molecules to study to understand cell physiology and disease pathogenesis. Previously, our lab and its collaborators used metabolic profiling in tandem with gene expression analysis and genotyping to construct a causal metabolite/transcript network in murine liver by which the metabolites glutamine/glutamate (Glx) influenced transcripts, including that encoding the gluconeogenic enzyme phosphoenolpyruvate carboxykinase 1 (Pck1). It was also shown that hepatic Glx concentrations and Pck1 expression are both elevated in diabetic BTBR *ob/ob* mice at 10 weeks of age relative to normoglycemic B6 *ob/ob* mice. From these preliminary data, we hypothesize that transcriptional regulation

of Pck1 by Glx may be important in explaining the upregulation of hepatic gluconeogenesis seen in type 2 diabetes.

To elucidate the significance and universality of this network, we have studied its role in rat hepatocytes and rat models of obesity (Zucker *fa/fa*) and diabetes (ZDF *fa/fa*). Here, we demonstrate that treatment of rat hepatocytes with 10 mM glutamine causes increased expression of Pck1 and intermediate transcript nodes in the Glx/Pck1 regulatory network, arginase 1 and alanine-glyoxylate aminotransferase, in lean Wistar rats. Hepatic Glx was markedly elevated in diabetic ZDF *fa/fa* rats when compared to both Zucker *fa/fa* rats and prediabetic ZDF *fa/fa* rats. Pck1 expression levels showed a strong trend to increase in diabetic ZDF *fa/fa* rats and prediabetic ZDF *fa/fa* rats relative to age-matched Zucker *fa/fa* rats. Our findings to date demonstrate that hepatic Glx levels are increased in two independent models of obesity-associated diabetes in rats and mice relative to nondiabetic obese controls. These increases in Glx may be related to increased Pck1 expression in both animal models that predisposes to poorly controlled hepatic glucose production and development of type 2 diabetes.

9:15 A.M.

A variant in long palate, lung, and nasal epithelium clone 1 is associated with cholera in a Bangladeshi population

REGINA LAROCQUE, M.D., Early Career Awardee, Massachusetts General Hospital

■ *Vibrio cholerae* causes a dehydrating diarrheal illness that can be rapidly fatal in the absence of specific treatment. The organism is an historic scourge and, like similar infectious diseases, may have influenced the evolution of the human genome. We report here the results of the first candidate gene association study of cholera.

In a family-based study of 76 pedigrees from Dhaka, Bangladesh, we evaluated the association between cholera and five candidate genes: cystic fibrosis transmembrane receptor; lactoferrin; long palate, lung, and nasal epithelium clone 1 (LPLUNC1); estrogen-related receptor α ; and calcium-activated chloride channel 1. We found a significant association with a marker in the promoter region of LPLUNC1 (rs11906665), a member of a family of evolutionarily conserved innate immunity proteins. A previous microarray-based study of duodenal biopsies revealed significantly increased expression of LPLUNC1 in cholera patients compared to healthy control subjects.

Our results suggest that variation in host innate immune responses may influence the outcome of exposure to *V. cholerae* in an endemic setting.

9:30 A.M.

Predicting HIV-1 RNA level and loss of virologic suppression among HIV type-1-infected children receiving antiretroviral therapy in Tanzania

SUSAN D. EMMETT, Medical Fellow, Duke University School of Medicine

Mentor: Nathan M. Thielman, M.D., Duke University School of Medicine

■ HIV RNA monitoring continues to be widely unavailable in resource-limited settings despite rapid expansion of antiretroviral therapy (ART), and reliability of clinical and immunologic data to predict virologic failure (VF) remains poorly understood. We identified predictors of VF to address this gap in monitoring capacity.

Children presenting for routine HIV care at a Tanzanian referral center who were 2–14 years of age and on ART for ≥ 6 months were randomly selected for enrollment. Clinical staging, social information, CD4, HIV RNA, and complete blood count (CBC) were collected at enrollment, and prior CD4 and CBC data were abstracted from charts. Predictors of failure (HIV RNA > 400 copies/ml) were determined using logistic regression.

Of 128 children enrolled to date (59 male, mean age 8.4 years, mean ART duration 2.5 years, median ART 2.7 years), 37 (29%) demonstrated VF. Physician documentation of maladherence (OR 4.5, $P=0.005$), but not family report of missed doses, was significantly associated with VF. Across 7 other psychosocial variables and 13 stage-two and -three conditions, there were no significant predictors of VF. Lower enrollment CD4% (OR=2.0, $P=0.002$, per 10-point difference [10pt]), lower minimum posttreatment CD4% (OR=2.2, $P<0.001$, 10pt), and lower maximum posttreatment CD4% (OR=1.6, $P=0.03$, 10pt) were each significantly predictive of VF. Lower enrollment platelet count (OR=1.8, $P=0.01$, 10pt) and lower maximum posttreatment hemoglobin (OR=1.4, $P=0.03$) were also significantly predictive of failure. Duration of therapy, ART regimen, and gender were not associated with VF.

This study demonstrates the success of long-term ART in a resource-limited setting, with $> 70\%$ of children virologically suppressed after a mean of 2.5 years on therapy. Preliminary analyses suggest that clinical staging is not useful in predicting VF. Our data provide laboratory parameters that may improve identification of children at risk for VF in settings where viral load monitoring is unavailable.



R. LAROCQUE



S. D. EMMETT

WEDNESDAY
Room D-124

9:45 A.M.

A prospective study of tooth loss and cancer risk in a cohort of male smokers

SAMANTHA JORDAN, Research Scholar, Tufts University School of Dental Medicine

Preceptor: Christian C. Abnet, Ph.D., National Cancer Institute, National Institutes of Health

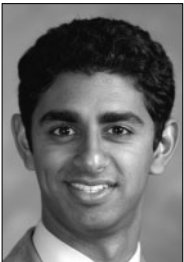
■ Tooth loss, a sign of poor oral health, has been associated with an increased risk of oral, esophageal, gastric, and pancreatic cancers in different populations. The goal of this study was to prospectively examine the association between tooth loss and cancer risk in the α -tocopherol, β -carotene cancer prevention (ATBC) study cohort. After excluding subjects with missing data, the final analytic sample included 26,960 male smokers who contributed 382,832 person-years of follow up.

Cox proportional hazards models, adjusted for age, smoking, alcohol, diet, education, marital status, denture use, serum cholesterol, body mass index, and physical activity, were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between tooth loss and cancer risk at 18 different cancer sites. Furthermore, we found significant interactions between tooth loss, denture use, and cancer risk at several sites, so we estimated cancer risk separately in strata of denture users and nonusers.

We found that tooth loss was associated with a change in risk for a number of different cancers. Overall, compared to subjects who had lost 10 or fewer teeth, edentulous subjects had an elevated risk for cancer. Edentulism was associated with a higher risk for lung cancer, the most frequent incident cancer. When divided by histology, the association with lung cancer became null for adenocarcinomas while remaining elevated for the other lung cancer subtypes. Stratification on denture use revealed a qualitative interaction with lung adenocarcinomas such that edentulism was associated with an increased risk in denture users, but a decreased risk in nonusers. We found increased cancer risk at several other sites, but also found that tooth loss appeared to confer lower cancer risk for other sites. Future studies are needed to elucidate the mechanism that underlies the association of tooth loss and cancer risk.



S. JORDAN



N. DESAI

10:00 A.M.

Molecular characterization of ductal carcinoma in situ

NEIL DESAI, Medical Fellow, Yale School of Medicine

Mentor: David F. Stern, Ph.D., Yale School of Medicine

■ Ductal carcinoma in situ (DCIS) constitutes a key step in the development of invasive ductal carcinoma (IDC), the most common form of invasive breast cancer. DCIS is thought to precede nearly all IDC lesions while also having accrued a majority of the genetic changes that will characterize its molecular phenotype if invasiveness emerges. However, little is known about the molecular factors defining risk for progression, risk for recurrence with and without treatment, or risk for a type of recurrence whether invasive or DCIS. These deficiencies are accentuated by the increased epidemiologic profile of DCIS, as screening mammography identifies earlier and smaller lesions whose natural histories are even less well defined.

We are investigating the possibility of identifying molecular signatures to stratify DCIS more effectively than current histopathological criteria. First, retrospective cohorts from archival formalin-fixed paraffin-embedded (FFPE) tissue with clinical follow-up are being used to compare 1) DCIS that does not recur to DCIS that does recur and 2) DCIS that recurs as local versus invasive lesions. Second, we will use a “mammosphere” cell culture model of DCIS from core biopsies prospectively to compare 1) DCIS with and without concurrent invasiveness at diagnosis and 2) DCIS component of DCIS with invasion to invasive component of the same specimen.

Our ongoing analyses utilize microarray for expression analysis validated by qRT-PCR, copy-number variation (CNV) array for genomic amplification/deletion event analysis, and selective resequencing of genes of interest to establish these molecular signatures. Further, to address issues in previous studies of specificity of tissue being analyzed in the retrospective cohort FFPE specimens, we will use laser capture microdissection (LCM) to isolate DCIS epithelium selectively.

It is our hope that these discovery studies will aid in the development of diagnostic and prognostic tools in DCIS as well as novel targets for therapeutic investigation.

10:15 A.M.

Patients with congenital heart disease and laterality defects exhibit ciliary dysfunction: a possible contributor to surgical outcomes?

RACHEL A. GIESE, Research Scholar, University of Texas Health Science Center at San Antonio

Preceptor: Cecilia W. Lo, Ph.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ The cilia of the embryonic node play an important role in the determination of left-right patterning and heart development. In the mouse model of primary ciliary dyskinesia (PCD), congenital heart disease (CHD) is present with and without heterotaxy. In a previous retrospective human study of postoperative morbidity and mortality in patients undergoing corrective surgery for heart disease, patients with heterotaxy have worse outcomes than case-matched controls without heterotaxy. We hypothesize that PCD may be the underlying etiology for this discrepancy, perhaps due to impaired mucociliary clearance or abnormal signaling. To determine the role of ciliary dyskinesia in CHD, we screened 45 patients with laterality abnormalities involving the heart with or without left-right patterning defects in other organs. Ciliary dysfunction was assessed by the analysis of exhaled nasal nitric oxide measurements, panel review of video microscopy of ciliary beat, electron microscopy of the ciliary axoneme, immunohistochemistry, clinical history, and gene mutation analysis. In the first cohort of 34 heterotaxy patients with laterality defects of the heart and abdominal organs or the lungs, 19 (56%) patients were classified as likely having ciliary dysfunction and 15 (44%) were deemed unlikely to have ciliary dysfunction. In the second cohort of 11 heterotaxy patients with isolated CHD and laterality defects of the heart alone, 3 (27%) patients were determined as likely to have ciliary dysfunction and 8 (73%) were unlikely candidates for ciliary dysfunction. These results indicate that defects associated with multiple organs are more likely associated with ciliary dysfunction. Postoperative outcomes such as length of hospital stay, length of time on the ventilator, rate of infection, medications administered, and respiratory complications are being studied to determine whether poor outcome may be linked with ciliary dysfunction.

10:30 A.M.

On-statin cholesteryl ester transfer protein mass and risk of recurrent coronary events: Results from the PROVE IT-TIMI 22 study

AMIT V. KHERA, Medical Fellow, University of Pennsylvania School of Medicine

Mentor: Daniel J. Rader, M.D., University of Pennsylvania School of Medicine

■ Although cholesteryl ester transfer protein (CETP) plays an important role in human lipoprotein metabolism, its relationship to coronary artery disease remains controversial. One potent CETP inhibitor was associated with increased rates of cardiovascular events in a large clinical trial despite dramatically increasing plasma high-density lipoprotein cholesterol (HDL-C) levels. CETP facilitates the transport of HDL-derived cholesterol to the liver, potentially promoting the reverse cholesterol transport (RCT) pathway in some patients. We evaluated the relationship of plasma CETP mass, measured after four months of statin therapy, and the risk of recurrent myocardial infarction or death from coronary causes among 3,218 patients following acute coronary syndromes in the PROVE IT-TIMI 22 study.

Our analysis indicated that 150 patients experienced a recurrent coronary event over a mean follow-up of 1.8 years. Increasing on-statin CETP mass was inversely related to risk of coronary events in both unadjusted (HR/SD increase 0.78; 95% CI 0.66–0.91; $p=0.002$) and fully adjusted (HR/SD increase 0.81; 95% CI 0.68–0.96; $p=0.016$) analyses that included traditional cardiovascular risk factors. A similar trend was observed across increasing CETP mass quartiles (p -trend=0.07). A significant interaction between CETP mass and on-treatment low-density lipoprotein cholesterol (LDL-C) was noted (p -interaction=0.007). CETP mass above the median was associated with decreased risk in patients with LDL-C below the median value of 80 mg/dl (HR 0.52; 95% CI 0.31–0.89; $p=0.02$), but not in patients with LDL-C greater than the median.

The relationship between CETP and coronary outcomes in this large clinical cohort varied according to patients' LDL-C levels. This finding is consistent with CETP facilitating RCT in the setting of robust LDL clearance and may have important implications for efforts to optimally target patients with pharmacologic CETP inhibition.



R. A. GIESE



A. V. KHERA

WEDNESDAY
Room D-125

9:15 A.M.

MTHFR* 677C→T disrupts prefrontal and dopaminergic function in schizophrenia*JOSHUA L. ROFFMAN, M.D.**, Early Career Awardee, Harvard Medical School

J. L. ROFFMAN

■ Schizophrenia remains one of the most disabling psychiatric disorders, and with a heritability approaching 80%, it also ranks among the most strongly genetic. Identification of risk-conferring genetic variants could provide valuable clues to the etiopathogenesis of schizophrenia, subsequently improving treatment. However, thus far, even the most promising of schizophrenia candidate genes have been associated with only modest effects at the level of clinical phenotypes, and pharmacogenetic advances remain equally elusive. We are using multimodal *in vivo* neuroimaging to amplify the signal of candidate genes, examining allelic effects at the levels of neural activation and dopamine receptor function.

We have focused on the *MTHFR* 677C→T polymorphism, a common, functional variant in the folate metabolic pathway that has been associated with consistent but small elevations in schizophrenia risk, as well as with comorbid executive dysfunction. Using functional magnetic resonance imaging (fMRI), we have observed robust, detrimental effects of the hypofunctional 677T allele on frontal lobe activation during executive function paradigms in two cohorts of schizophrenia patients, but not in healthy participants. These findings strengthen when they are considered in concert with the *COMT* 158Val→Met polymorphism, pointing to dysfunctional prefrontal dopamine signaling as a mechanism for *MTHFR* effects in schizophrenia. Supporting this idea, preliminary data from positron emission tomography (PET) studies involving the D1-specific ligand [¹¹C] NNC 112 correlate 677T allele load with reduced prefrontal dopamine tone.

In the context of recent postmortem data identifying reduced *COMT* promoter methylation in prefrontal cortex of schizophrenia patients, these findings converge around a model implicating the 677T allele in increased *COMT* expression and subsequent deficiencies in prefrontal dopamine signaling. Ongoing work will determine whether this mechanism underlies *MTHFR* effects on neuroimaging and clinical phenotypes. This approach may guide the identification of schizophrenia patients who are most likely to benefit from novel dopamine and folate augmentation strategies.



M. VESTAL

9:30 A.M.

Why little Sally can't pay attention in class: functional neuroimaging of interictal attention deficits in childhood absence epilepsy**MATTHEW VESTAL**, Medical Fellow, Yale School of Medicine

Mentor: Hal Blumenfeld, M.D., Ph.D., Yale School of Medicine

■ Childhood absence epilepsy (CAE) is a significant cause of impaired attention and social dysfunction in school-age children. Absence seizures resemble staring spells and last 5–10 seconds during which children are unresponsive to external stimuli. CAE affects 10–15% of children with epilepsy, and seizures can occur up to hundreds of times per day. Even if seizure control is possible through medication, many children suffer from impaired attention between absence episodes. The mechanisms of impaired attention during (ictal) and between (interictal) absence seizures are not known. Since most children with CAE achieve sufficient seizure control, the majority of their deficits occur interictally, and it is imperative that the mechanisms of these deficits be elucidated. In this study, we examine interictal attention deficits in children with CAE using functional magnetic resonance imaging (fMRI) during a continuous performance task (CPT) of attentional vigilance.

To evaluate interictal attentional vigilance, we use a CPT where children (ages 6–18 years) are asked to watch a string of letters and press a button whenever they see the letter “X.” During this CPT, we monitor fMRI signal changes with a 3-Tesla scanner, using these changes as an indicator of changes in focal brain activity associated with the attention task. We compare the changes in fMRI signal during the attention task in children with CAE to the changes that occur in a cohort of control children that is matched for age, sex, intelligence quotient, and socioeconomic status.

Here, we show that children with CAE have significantly higher error rates during CPT than control subjects. Additionally, we observe differences in patterns of brain activity in children with CAE (compared to controls), specifically in areas important for attentional vigilance. From this, we conclude that focal dysfunction in selective corticothalamic regions, normally important for attention, may cause interictal attention deficits in CAE.

9:45 A.M.

Characterization of Neuregulin 3 (NRG3) and its role in schizophrenia**WEE-TIN KAO**, Research Scholar, School of Medicine at Stony Brook University Medical Center

Preceptor: Daniel R. Weinberger, M.D., National Institute of Mental Health, National Institutes of Health

■ Schizophrenia is characterized by a range of symptoms from psychosis, disorganization, and mania to negative symptoms, catatonia, and depression. Many etiologies for the disease have been explored, and it has become apparent that genetics plays a significant role. Current theories suggest that small variations in many genes can confer an increased total risk for schizophrenia. Genomewide linkage studies have implicated chromosome 10q22 as a susceptibility locus. Fine-mapping studies have identified a gene, Neuregulin 3 (NRG3), in this region as a risk gene. NRG3 has an EGF domain and acts as a ligand for ErbB4, a receptor tyrosine kinase. It is involved in oligodendrocyte survival and cell fate decisions of pluripotent epidermal cell populations. Numerous association studies have identified a pool of SNPs that seem to confer increased risk for schizophrenia, and a recent study identified a fetal variant of NRG3 containing three unique exons. In this study, we have identified new variants of the NRG3 gene.

Adult human brain cDNA libraries were generated using total RNA from hippocampal and reference brain samples. A series of forward and reverse primers were designed. The gene was amplified, cloned, and sequenced. We report the presence of the fetal variant in the adult brain. We were also able to identify two novel exons, E8A and E6A. An additional finding is an elongated exon1A containing an extra 75 base pairs flanked by a GT dinucleotide at the 5' end and an AG dinucleotide at the 3' end consistent with a splice donor and acceptor site. The inclusion of this elongated exon disturbs the reading frame. Its presence may be required for mRNA stability, efficiency, or trafficking of the mRNA transcript within the cell. We are currently conducting qPCR studies to quantify these variants in human postmortem brain tissues collected at the Clinical Brain Disorders Branch of the National Institute of Mental Health.

10:00 A.M.

Characterization of PCDH7 and GJA1 in the formation of brain metastases**KIMBERLEY S. MAK**, Medical Fellow, Harvard Medical School

Mentor: Joan Massagué, Ph.D., Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center

■ Brain metastasis is the most common intracranial malignancy in adults, affecting ~10% of cancer patients. Its poor prognosis and neurologic consequences render it a critically important area of research. Using in vivo selection, the Massagué laboratory has isolated brain metastatic (BrM) cell populations from breast cancer patients and identified a gene-expression profile associated with the brain metastatic phenotype.

To dissect the molecular mechanisms of brain metastasis, we focused on two of the genes more highly expressed in BrM cells: protocadherin 7 (*PCDH7*), an adhesion molecule predominantly expressed in brain and heart, and gap junction protein alpha 1, 43 kDa (*GJA1*, also called *connexin 43*), which is abundantly expressed in astrocytes. We hypothesized that expression of these molecules in brain metastatic cells mediates communication with brain endothelial or glial cells, facilitating cancer cell infiltration and survival in the brain.

We generated BrM cell lines with PCDH7 or GJA1 downregulation using RNAi. Initial in vivo experiments revealed that BrM cells with PCDH7 knockdown, but not GJA1 knockdown, exhibited decreased brain metastatic activity compared to control BrM cells. Ongoing in vivo studies will characterize the phenotype of these metastatic lesions. We performed in vitro assays to evaluate adhesion of tumor cells to brain endothelium and transmigration through the blood-brain barrier (BBB). Downregulation of PCDH7 or GJA1 did not affect the adhesive properties of BrM cells, suggesting that these genes are not singly required for adhesion to the brain endothelium under these conditions. In contrast, knock down of PCDH7 but not GJA1 resulted in decreased BBB transmigration.

Our results indicate that PCDH7 mediates brain metastasis in part by collaborating in BBB extravasation. The role of GJA1 in brain metastasis remains undefined, although this gene may be acting in combination with others. Tumor cell-astrocyte coculture studies are being conducted to evaluate the role of these genes in tumor cell survival and growth upon brain infiltration.



W. KAO



K. S. MAK

WEDNESDAY
Room D-125

10:15 A.M.

Emboli extravasation is an alternative mechanism for cerebral microvascular recanalization

CARSON K. LAM, Medical Fellow, Northwestern University, The Feinberg School of Medicine

Mentor: Jaime Grutzendler, M.D., Northwestern University, The Feinberg School of Medicine

■ Cerebral microvascular occlusion is a common phenomenon that frequently remains asymptomatic and could thus be an underappreciated mechanism of brain pathology. The majority of occlusive events are rapidly cleared by hemodynamic forces and the fibrinolytic system, resulting in no apparent residual damage. However, recurring occlusions or failure to recanalize microvessels may lead to disruption of brain circuits and significant functional deficits. We investigated the natural course and consequences of cerebral capillary and terminal arteriole occlusions by imaging fluorescent microemboli in fixed tissues and the living mouse brain with confocal and two-photon microscopy. Surprisingly, the majority of emboli that failed to be washed out or lysed within ~24 hours were found to translocate to the extravascular space within 2–5 days, leading to reestablishment of blood flow, and were subsequently phagocytosed by microglia. The extravasation process involved a coordinated series of events, including focal microvascular constriction and extrusion of emboli via a transient breach in the blood-brain barrier, and was markedly inhibited by a matrix metalloproteinase 2/9 inhibitor. Although microvascular occlusion was not associated with cell death, it led to focal synapse loss that recovered after vessel recanalization. In aged mice, however, extravasation was markedly delayed, resulting in more persistent tissue hypoxia, synaptic damage, and perivascular cell death. Thus, our study identifies a novel cellular mechanism that is critical for the recanalization of occluded microvessels, which fail to be cleared by the fibrinolytic system. Alterations in the efficiency of this protective mechanism may contribute to microvascular pathology and age-related cognitive decline.



C. K. LAM



C. C. ZYGOURAKIS

10:30 A.M.

Downstream mediators of SOX6 control over cortical interneuron development

CORINNA CLIO ZYGOURAKIS, Medical Fellow, Harvard Medical School

Mentor: Jeffrey D. Macklis, M.D., Harvard Medical School, Massachusetts General Hospital

■ The mammalian neocortex is an extraordinarily complex and heterogeneous system composed of hundreds of distinct neuronal subtypes. To produce this heterogeneity, spatially and temporally segregated molecular signals direct the generation of diverse neuronal subtypes. Inhibitory GABAergic interneurons are a broad neuronal subclass that modulates cortical network activity, and their dysfunction has been implicated in a number of developmental disorders, including epilepsy, autism, and schizophrenia. These neurons arise from (“subpallial”) telencephalic embryonic structures (MGE, CGE) and comprise ~20% of neocortical neurons, exhibiting remarkable subtype diversity across morphological, physiological, and molecular classes. Recently, members of our lab showed that the SRY-box transcription factor SOX6 controls the development of cortical interneuron subtype diversity by regulating multiple aspects of neuronal differentiation during development.

To identify potential downstream targets (direct or indirect) of SOX6 function during interneuron differentiation, I performed microarray differential gene expression analysis, in collaboration with graduate student Eiman Azim, of wild-type and *Sox6*^{-/-} newborn cortical interneurons (taken from the ventral telencephalon at 13.5 days postconception). Statistical analyses with multiple normalization methods ($P < 0.005$, absolute fold change > 1.5) revealed hundreds of genes that are differentially expressed between WT and *Sox6*^{-/-} interneurons. A combination of approaches (including computational transcription factor motif scanning for SOX binding sites, as well as literature and online database mining for gene function and developmental gene expression profiles) refined this gene list to approximately 30 high-priority candidates. Of these candidate genes, we selected four top-priority candidate transcription factors; their expression coincides spatially and temporally with interneuron development (as confirmed via *in situ* hybridization and/or immunocytochemistry). We are currently performing gain- and loss-of-function analysis using *in utero* electroporation of overexpression constructs, as well as loss-of-function transgenic mouse lines, respectively, in order to identify any control these genes may have over cortical interneuron subtype differentiation and diversity.

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Name	Day	Time	Room	Page
Azodi, Shila	Tuesday	6:30–7:30 p.m.	Atrium	85
Bahadu, Shaka J.D.	Monday	3:00–4:00 p.m.	Atrium	58
Baharanyi, Hasani K.	Tuesday	6:30–7:30 p.m.	Atrium	84
Bansal, Amar D.	Monday	10:15 a.m.	D-124	32
Batra, Ayush	Tuesday	11:45 a.m.	D-125	77
Beaudry, Steven	Tuesday	9:00 a.m.	D-124	66
Blaine, Kevin P.	Monday	3:00–4:00 p.m.	Atrium	52
Blake, Patrick W.	Monday	4:00–5:00 p.m.	Atrium	64
Boonyaratanakornkit, Jim B.	Tuesday	9:45 a.m.	D-124	68
Bosse, Kristopher	Monday	9:15 a.m.	Auditorium	43
Braunstein, Lior	Monday	11:30 a.m.	D-124	34
Browning, Rebekah	Monday	4:00–5:00 p.m.	Atrium	62
Buchanan, Ian M.	Monday	4:00–5:00 p.m.	Atrium	59
Cai, Ann	Monday	4:00–5:00 p.m.	Atrium	62
Caretto, David Christopher	Tuesday	11:00 a.m.	D-124	70
Castillo, Marianne D.	Monday	4:00–5:00 p.m.	Atrium	65
Chan, Justin	Tuesday	9:30 a.m.	D-124	67
Chan, Yvonne R.	Monday	1:45 p.m.	Auditorium	50
Chang, John T.	Monday	1:30 p.m.	Auditorium	50
Chang, Jessica	Tuesday	6:30–7:30 p.m.	Atrium	88
Chao, Mark P.	Monday	12:15 p.m.	Auditorium	49
Chard, Rachel L.	Monday	4:00–5:00 p.m.	Atrium	60
Chen, Jennifer K.	Monday	4:00–5:00 p.m.	Atrium	65
Chen, Jenny	Monday	3:00–4:00 p.m.	Atrium	56
Cheng, Rex G.	Monday	3:00–4:00 p.m.	Atrium	55
Chong, H. Jonathan	Tuesday	9:00 a.m.	D-125	72
Chu, Lisa L.	Monday	9:45 a.m.	D-124	31
Chun, Hyung J.	Monday	10:45 a.m.	D-125	39
Clavijo, Raul I.	Tuesday	7:30–8:30 p.m.	Atrium	92
Cohen, Justin Brent	Tuesday	9:15 a.m.	Auditorium	79
Condren, Audree B.	Monday	11:30 a.m.	D-125	40
Cook, MacKenzie R.	Monday	9:45 a.m.	D-125	37
Czechowicz, Agnieszka	Tuesday	9:00 a.m.	Auditorium	78
Davis, Jeremiah C.	Tuesday	11:45 a.m.	D-124	71
Desai, Neil	Wednesday	10:00 a.m.	D-124	98
Emmett, Susan D.	Wednesday	9:30 a.m.	D-124	97
Ene, Chiba	Monday	4:00–5:00 p.m.	Atrium	60
Fehniger, Todd A.	Monday	2:15 p.m.	Auditorium	51
Fewkes, Natasha	Tuesday	9:30 a.m.	Auditorium	79
Fowler, Cedar J.	Tuesday	9:45 a.m.	Auditorium	80

INDEX OF PRESENTATION TIMES

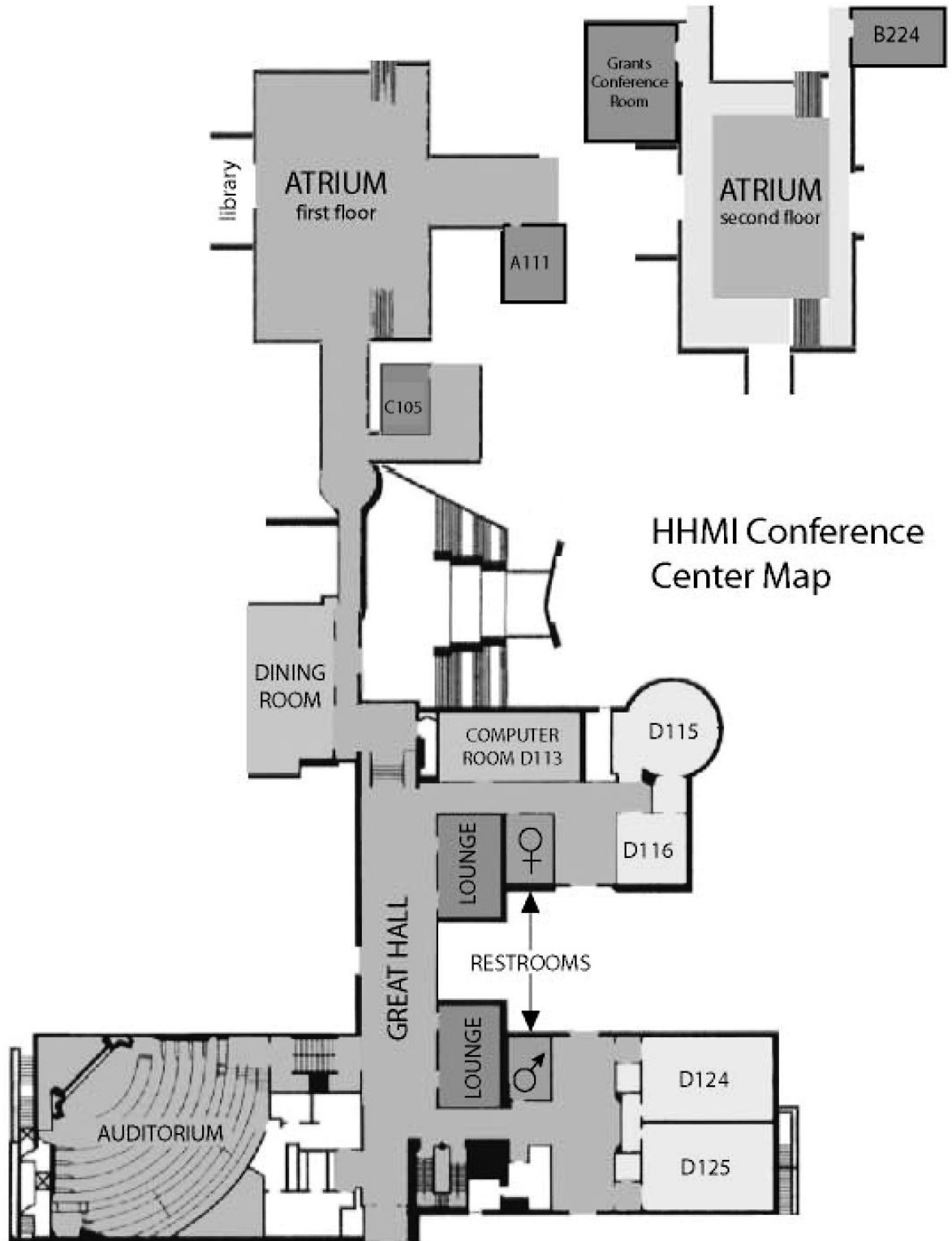
Name	Day	Time	Room	Page
Frazer, Lauren	Monday	3:00–4:00 p.m.	Atrium	53
Galvez, Michael G.	Monday	11:00 a.m.	D-125	39
Giacomini, Craig P.	Monday	9:15 a.m.	D-124	30
Giese, Rachel A.	Wednesday	10:15 a.m.	D-124	99
Goldstein, Matthew J.	Monday	Noon	Auditorium	48
Gordon, Joshua A.	Tuesday	9:15 a.m.	D-125	73
Graham, Timothy E.	Monday	9:00 a.m.	D-125	36
Green, Ari	Tuesday	10:30 a.m.	D-125	75
Guenther, Lillian M.	Monday	10:15 a.m.	D-125	38
Gupta, Divakar	Tuesday	7:30–8:30 p.m.	Atrium	96
Halawi, Mohamad	Tuesday	9:30 a.m.	D-125	73
Han, Seunggu J.	Monday	9:30 a.m.	Auditorium	44
Harel, Asaff	Tuesday	10:45 a.m.	Auditorium	81
Hawkes, Jason E.	Monday	4:00–5:00 p.m.	Atrium	63
He, Lucy Le	Monday	10:00 a.m.	D-125	38
Helmy, Karim Y.	Monday	4:00–5:00 p.m.	Atrium	59
Hillesland, Heidi	Tuesday	9:15 a.m.	D-124	67
Hiniker, Susan M.	Monday	10:00 a.m.	Auditorium	45
Hobson, Suejy	Tuesday	10:45 a.m.	D-124	69
Hodavance, Michael	Tuesday	7:30–8:30 p.m.	Atrium	94
Hong, Jennifer	Tuesday	11:30 a.m.	D-125	77
Hsieh, Fred H.	Tuesday	8:45 a.m.	Auditorium	78
Hsu, Jeffrey J.	Tuesday	10:00 a.m.	D-125	74
Huss, Ryan Steven	Monday	3:00–4:00 p.m.	Atrium	54
Jagger, Brett	Monday	3:00–4:00 p.m.	Atrium	56
Ji, Hanlee P.	Monday	9:00 a.m.	D-124	30
Johnson, Timothy Van	Monday	4:00–5:00 p.m.	Atrium	63
Jonelis, Michelle Binder	Tuesday	6:30–7:30 p.m.	Atrium	90
Jones, Guy C.	Monday	9:30 a.m.	D-124	31
Jordan, Samantha	Wednesday	9:45 a.m.	D-124	98
Kalbasi, Anusha	Monday	11:45 a.m.	Auditorium	48
Kao, Wee-Tin	Wednesday	9:45 a.m.	D-125	101
Kasowski, Maya	Tuesday	7:30–8:30 p.m.	Atrium	95
Keaton, Amelia	Tuesday	11:00 a.m.	Auditorium	82
Khachi, Steve	Tuesday	10:45 a.m.	D-125	75
Khera, Amit V.	Wednesday	10:30 a.m.	D-124	99
Kosztowski, Thomas Adam	Monday	11:15 a.m.	Auditorium	47
Lam, Carson K.	Wednesday	10:15 a.m.	D-125	102
LaRocque, Regina	Wednesday	9:15 a.m.	D-124	97
Lee, Angela Catherine	Monday	11:15 a.m.	D-125	40

INDEX OF PRESENTATION TIMES

Name	Day	Time	Room	Page
Lewis Jr., John Strudwick	Tuesday	9:45 a.m.	D-125	74
Liu, Christopher Cheng-Yu	Tuesday	6:30–7:30 p.m.	Atrium	88
Ma, Gene Kew	Monday	Noon	D-124	35
Mabardy, Allan S.	Monday	10:00 a.m.	D-124	32
Mak, Kimberley S.	Wednesday	10:00 a.m.	D-125	101
Malik, Priya	Monday	11:00 a.m.	D-124	33
McEvoy, Sean	Tuesday	11:00 a.m.	D-125	76
Mendel, J. Brett	Monday	3:00–4:00 p.m.	Atrium	55
Méndez, Eduardo	Tuesday	10:30 a.m.	Auditorium	81
Meoli, Elise M.	Tuesday	11:15 a.m.	D-124	70
Minear, Steve	Tuesday	7:30–8:30 p.m.	Atrium	91
Modi, Yasha	Monday	11:45 a.m.	D-124	35
Narendra, Derek Paul	Tuesday	6:30–7:30 p.m.	Atrium	86
Narla, Goutham	Monday	9:15 a.m.	D-125	36
Nayak, Nikhil R.	Tuesday	6:30–7:30 p.m.	Atrium	87
Nuyujukian, Paul	Tuesday	6:30–7:30 p.m.	Atrium	89
Onaitis, Mark	Monday	9:00 a.m.	Auditorium	43
Parker, Rell	Tuesday	6:30–7:30 p.m.	Atrium	86
Pasricha, Sarina	Tuesday	11:15 a.m.	Auditorium	82
Pearl, Jeremy	Tuesday	10:00 a.m.	Auditorium	80
Perlmutter, David Henry	Tuesday	6:30–7:30 p.m.	Atrium	85
Poling, Justin	Monday	Noon	D-125	41
Pomerantz, Rebecca G.	Monday	3:00–4:00 p.m.	Atrium	54
Puri, Tipu S.	Tuesday	10:30 a.m.	D-124	69
Purow, Benjamin	Monday	10:45 a.m.	Auditorium	46
Reilley, Matthew J.	Monday	11:45 a.m.	D-125	41
Robinson, Makeda L.	Monday	3:00–4:00 p.m.	Atrium	57
Roffman, Joshua L.	Wednesday	9:15 a.m.	D-125	100
Romesser, Paul B.	Monday	4:00–5:00 p.m.	Atrium	61
Rusk, Sarah Elizabeth	Tuesday	7:30–8:30 p.m.	Atrium	93
Ryder, Alex	Tuesday	10:00 a.m.	D-124	68
Samuel, Michelle	Monday	4:00–5:00 p.m.	Atrium	64
Schneider, Logan	Tuesday	6:30–7:30 p.m.	Atrium	89
Shearin, Abigail	Tuesday	11:45 a.m.	Auditorium	83
Shih, Hubert	Monday	11:15 a.m.	D-124	34
Sifri, Costi	Monday	2:00 p.m.	Auditorium	51
Staser, Karl William	Monday	3:00–4:00 p.m.	Atrium	52
Steward-Tharp, Scott	Monday	3:00–4:00 p.m.	Atrium	53
Sulyanto, Rosalyn M.	Tuesday	7:30–8:30 p.m.	Atrium	91
Taylor, Tina D.	Monday	10:15 a.m.	Auditorium	45

INDEX OF PRESENTATION TIMES

Name	Day	Time	Room	Page
Taraska, Corinne	Monday	11:30 a.m.	Auditorium	47
Thawani, Jayesh P.	Tuesday	6:30–7:30 p.m.	Atrium	87
Tokita, Mari Johanna	Tuesday	7:30–8:30 p.m.	Atrium	92
Toprani, Sheela	Tuesday	11:15 a.m.	D-125	76
Tsung, Allan	Monday	10:45 a.m.	D-124	33
Valdez, Jessica M.	Monday	3:00–4:00 p.m.	Atrium	57
Varley, Patrick	Tuesday	11:30 a.m.	Auditorium	83
Vestal, Matthew	Wednesday	9:30 a.m.	D-125	100
Villarreal Jr., Guadalupe	Tuesday	7:30–8:30 p.m.	Atrium	95
Wang, Dean	Tuesday	7:30–8:30 p.m.	Atrium	93
Wang, Frederick	Monday	4:00–5:00 p.m.	Atrium	61
Weiner, Joshua I.	Tuesday	11:30 a.m.	D-124	71
Wellnitz, Kari A.	Tuesday	7:30–8:30 p.m.	Atrium	94
Wendte, Jered M.	Tuesday	8:45 a.m.	D-124	66
Williams, Emily P.	Monday	9:30 a.m.	D-125	37
Yazdani, Sima	Tuesday	6:30–7:30 p.m.	Atrium	84
Yeager, Caroline	Monday	12:15 p.m.	D-125	42
Yu, Paul B.	Tuesday	8:45 a.m.	D-125	72
Zaidi, Hasan A.	Monday	11:00 a.m.	Auditorium	46
Zumsteg, Zachary	Monday	9:45 a.m.	Auditorium	44
Zygourakis, Corinna Clio	Wednesday	10:30 a.m.	D-125	102



HHMI Conference Center Map

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