

2008

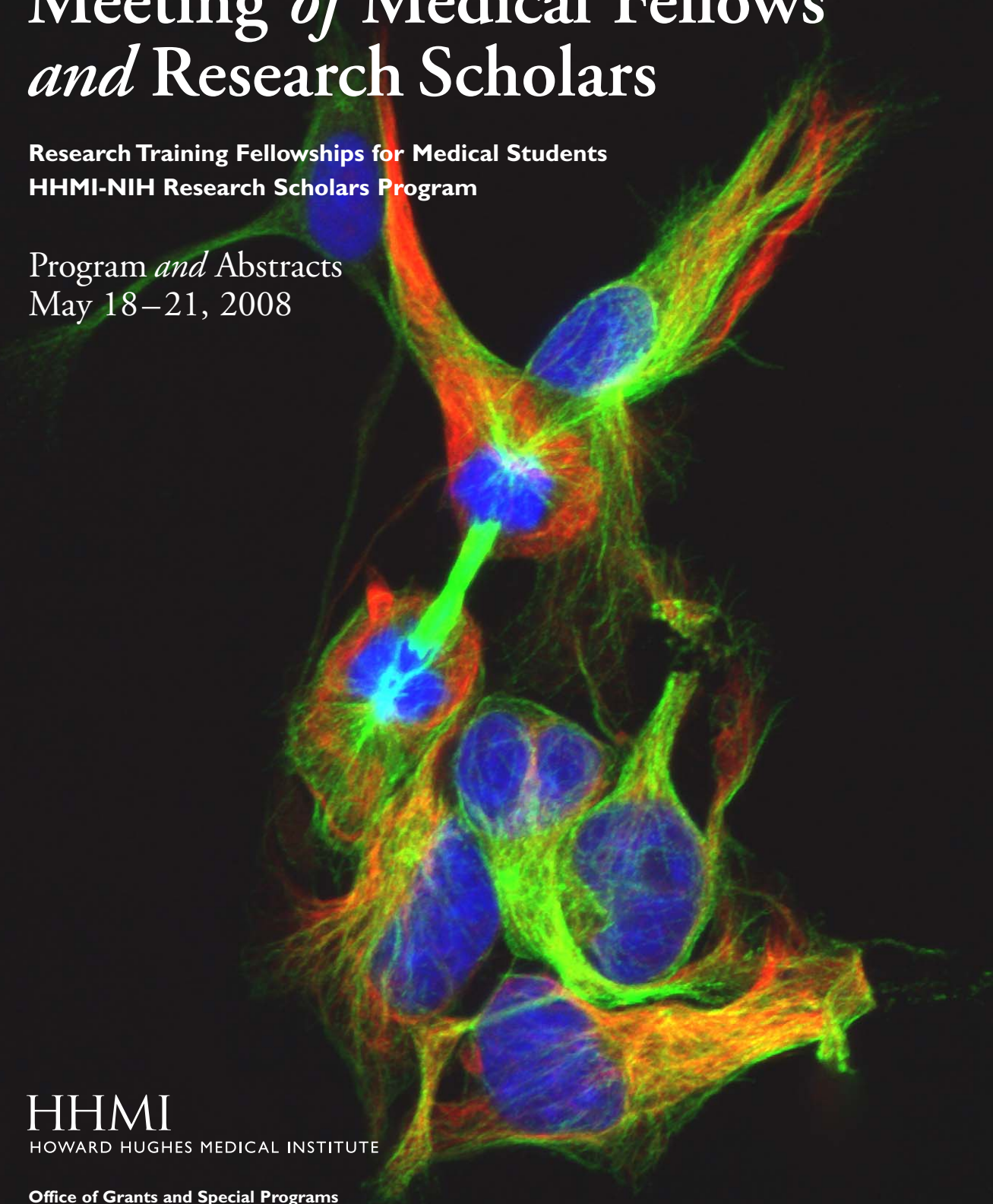
Meeting *of* Medical Fellows *and* Research Scholars

Research Training Fellowships for Medical Students
HHMI-NIH Research Scholars Program

Program *and* Abstracts
May 18–21, 2008

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Office of Grants and Special Programs



3	Introduction
4	Program Schedule
6	Keynote Speaker
7	Schedule of Presentations
19	Abstracts of Presentations
83	Howard Hughes Medical Institute Trustees Officers Principal Staff Members Office of Grants and Special Programs
85	Index of Presentation Times

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Cover: Glioblastoma multiforme (GBM) is the most common type of primary malignant brain tumor, with an average survival time of little more than a year despite aggressive multimodality treatments. Recent data suggest that many GBMs possess a subpopulation of cells termed tumor-initiating/tumor stem cells (TSCs) that have some of the hallmarks of normal neural stem cells (NSCs), including CD133 expression and differentiation potential. Understanding the differences in genomic and epigenetic regulation of differentiation between TSCs and normal NSCs may be important not only for fully understanding the biology of GBMs, but also for ultimately developing better therapies.

The cover shows a confocal micrograph of differentiating TSCs expressing both a neuron-specific marker, β -tubulin III (TUJ1; green), and an astrocyte-specific marker, glial fibrillary acid protein (GFAP; red). Nuclei (blue) are stained with DAPI. See abstract on page 63. (*Courtesy of Chiba Ene, HHMI-NIH research scholar, Neuro-Oncology Branch, NCI, NIH. Mentor: Howard A. Fine, M.D.*)

INTRODUCTION

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

In 1985, HHMI launched the HHMI-NIH Research Scholars Program in partnership with the National Institutes of Health. This program was established 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 launched 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.

Since the inception of the programs, HHMI has supported more than 2,000 medical fellows and research scholars. This year, 72 medical fellows and 48 research scholars will be presenting their research. This book contains the schedule and abstracts of their presentations.

In addition, 20 awardees of the 2007 HHMI Physician-Scientist Early Career Awards are participating in the meeting this year. The 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 presenting their research, participating in a career panel discussion on Monday evening, and co-chairing presentation sessions.

We are delighted to have Edwin M. Stone, M.D., Ph.D., as our honored speaker this year. Dr. Stone is an HHMI investigator, the Seamans-Hauser Chair in Molecular Ophthalmology, and professor of ophthalmology at the University of Iowa Roy J. and Lucille A. Carver College of Medicine. He will present some of his laboratory's findings that provide insight into gene replacement therapy for inherited eye diseases.

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 a bit better. In keeping with this objective, we have provided several opportunities for you to interact and network with each other in informal settings.

Another way for you to continue your association with HHMI and fellow trainees is through the HHMI Alumni Network, which is comprised of current and former awardees. Local networks have

been established in Boston, Northern California, Washington, D.C./Baltimore, Southern California, the Midwest, 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 a number of former medical student trainees, we are impressed by the pivotal effect that this research opportunity has had on their career development. We hope that you will view your training 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 and Research Scholars Programs in general. Please direct feedback regarding the Medical Fellows Program to Melanie Daub (daubm@hhmi.org) and feedback regarding the Research Scholars Program to Min Lee (leemin@hhmi.org).

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

Thomas R. Cech, 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

2008 MEETING OF MEDICAL FELLOWS AND RESEARCH SCHOLARS
HHMI HEADQUARTERS AND CONFERENCE CENTER, CHEVY CHASE, MARYLAND

Sunday, May 18, 2008

- 5:30–6:00 p.m. **Welcoming Reception**, *Great Hall*
- 6:00–7:00 p.m. Dinner, *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**, *Auditorium*
Peter J. Bruns, Ph.D., Vice President, Grants and Special Programs,
Howard Hughes Medical Institute
- Early Career Awardees Introduction**
- Panel Discussion: Pathway to Becoming a Physician-Scientist**
- Rathskeller open until 10:30 p.m.
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Monday, May 19, 2008

- 8:00 a.m. Breakfast, *Dining Room*
- 9:00–10:45 a.m. **Platform Presentations**
Immunology and Microbiology I, *Room A*
Biomedical Engineering, Biochemistry, and Bioinformatics, *Room B*
Cancer Biology I, *Auditorium*
- 10:45–11:00 a.m. Break, *Great Hall*
- 11:00 a.m. **Keynote Speaker**, *Auditorium*
*The Role of Clinician-Scientists in the Development of Gene Replacement
Therapy for Inherited Blindness*
Edwin M. Stone, M.D., Ph.D., Investigator, Howard Hughes Medical Institute;
Seamans-Hauser Chair in Molecular Ophthalmology and Professor of Ophthalmology,
University of Iowa Roy J. and Lucille A. Carver College of Medicine
- Noon Lunch, *Dining Room and Rathskeller*
- 1:30–3:15 p.m. **Platform Presentations**
Immunology and Microbiology II, *Room A*
Neuroscience I, *Room B*
Cancer Biology II, *Auditorium*
- 3:15–3:30 p.m. Break
- 3:30–4:15 p.m. **Poster Session A**, *Atrium*
- 4:15–5:00 p.m. **Poster Session B**, *Atrium*
- 5:00–5:30 p.m. **Reception**, *Great Hall*
- 5:30–6:30 p.m. Dinner, *Dining Room*
- 6:30 p.m. **Early Career Awardees' Presentations**, *Auditorium*

Rathskeller open until 10:30 p.m.
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Tuesday, May 20, 2008

- 7:30 a.m. Breakfast, *Dining Room*
- 8:30 a.m. **Platform Presentations**
 Molecular Biology, *Room A*
 Neuroscience II, *Room B*
 Immunology and Microbiology III, *Room B*
 Cancer Biology III, *Auditorium*
- 10:15–10:30 a.m. Break, *Great Hall*
- 10:30–Noon **Women Physician-Scientists Panel Discussion, Auditorium**
- Noon Box Lunch Pickup, *Dining Room*
- 12:30 p.m. **Depart for Social/Networking Event**
- 5:30–6:15 p.m. **Poster Session C, Cloister**
- 6:15–7:00 p.m. **Poster Session D, Cloister**
- 7:00 p.m. **Barbecue Dinner, Cloister**
- Return to HHMI Headquarters**
- Rathskeller open until 10:30 p.m.

Wednesday, May 21, 2008

- 8:00 a.m. Breakfast, *Dining Room*
- 9:00 a.m. **Platform Presentations**
 Cell and Developmental Biology, *Room A*
 Epidemiology, *Room B*
 Genetics, *Room B*
- 10:45 a.m. **Medical Fellows' Assembly, Auditorium**
- 10:45–11:00 a.m. Scholars' Break
- 11:00 a.m. **Recognition Ceremony, Auditorium**
- Opening Remarks**
 William R. Galey, 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**
 Thomas R. Cech, Ph.D., President, Howard Hughes Medical Institute
- Presentation of Fellows' Certificates**
- Noon Lunch, *Dining Room and Rathskeller*
- 1:30 p.m. Adjournment

The Role of Clinician-Scientists in the Development of Gene Replacement Therapy for Inherited Blindness

EDWIN M. STONE, M.D., PH.D.

Investigator, Howard Hughes Medical Institute; Seamans-Hauser Chair in Molecular Ophthalmology and Professor of Ophthalmology, University of Iowa Roy J. and Lucille A. Carver College of Medicine

■ Mutations in hundreds of different genes have been associated with inherited eye disease in humans, and many of these genes are expressed in the retina, the multilayered neuronal structure that converts light falling upon it into action potentials that travel through the optic nerve to the brain. The retina is surgically accessible in living human patients, which makes it possible to treat some inherited forms of blindness with viral-mediated gene replacement. Clinician-scientists have played, and will continue to play, a number of critical roles in the development of gene replacement therapy for inherited blindness, including gene discovery, genotype-phenotype correlation, elucidation of disease mechanisms, creation and characterization of animal models, large-scale genetic testing, and the design and execution of human clinical trials. This presentation will include specific examples of each of these kinds of experiments, highlighting the current multi-institutional effort to treat RPE65-associated Leber congenital amaurosis (blindness at birth) with AAV-mediated gene transfer. The talk will also touch on some of the personal, philosophical, and strategic aspects of large-scale scientific initiatives that often prove to be just as important to the ultimate success of such endeavors as the science itself. For example, the value of a favorable balance between leadership and team membership, collaboration and competition, academia and business, large labs and small labs, self and group will be discussed.



Edward Hefron

Dr. Stone is an HHMI investigator, the Seamans-Hauser Chair in Molecular Ophthalmology, and professor of ophthalmology at the University of Iowa Roy J. and Lucille A. Carver College of Medicine. He received his Ph.D. in cell biology and an M.D. degree from Baylor College of Medicine. Dr.

Stone completed a residency in ophthalmology and a fellowship in vitreoretinal diseases and surgery at the University of Iowa. His research is focused on inherited eye diseases, with special interest in locating and characterizing genes that are involved in macular degeneration, glaucoma, and photoreceptor degeneration (retinitis pigmentosa). In collaboration with Val Sheffield, M.D., Ph.D. (HHMI investigator, University of Iowa), he identified a number of genes responsible for important human eye diseases and characterized these in large international patient populations. In addition to his clinical responsibilities, Dr. Stone has undertaken an ambitious database project, Project 3000, which aims to collect information on all of the approximately 3,000 people in the United States with Leber congenital amaurosis, a rare genetic cause of blindness. He is also very interested in strategies for bringing new genetic discoveries to the clinic as rapidly as possible.

SCHEDULE OF PRESENTATIONS

MONDAY
ROOM A

Immunology and Microbiology I

page 20

- 9:00 a.m.** Live imaging of natural regulatory T cells during *Leishmania major* infection
David Chou, University of Pittsburgh School of Medicine (Ronald N. Germain, M.D., Ph.D., and Yasmine Belkaid, Ph.D.)
- 9:15 a.m.** Role of Rab11a and Rab11b GTPases in T cell receptor trafficking
Steven Brauer, Harvard Medical School (Lawrence E. Samelson, M.D.)
- 9:30 a.m.** Effector and regulatory T lymphocyte populations as determinants of autoimmunity in a model of systemic inflammatory disease
David Christopher Caretto, University of California, San Francisco, School of Medicine (Abul K. Abbas, M.D.)
- 9:45 a.m.** Assessing the capacity of bone marrow-derived plasmacytoid and myeloid dendritic cells to promote graft survival via induction of regulatory T cells
Eric Gehrie, Mount Sinai School of Medicine of New York University (Jonathan S. Bromberg, M.D., Ph.D.)
- 10:00 a.m.** Solution to a long-standing paradox? Disproportionate proliferation of the indirect pathway during acute rejection
Harras Zaid, University of California, San Francisco, School of Medicine (Sang-Mo Kang, M.D., and Qizhi Tang, Ph.D.)
- 10:15 a.m.** Genomic features contributing to chromosomal translocations
Jason M. Baron, Washington University School of Medicine (Barry Sleckman, M.D., Ph.D.)
- 10:30 a.m.** Ex vivo dynamic imaging of retinal microglia using time-lapse confocal microscopy
Jung Eun Lee, Duke University School of Medicine (Emily Chew, M.D., and Wai T. Wong, M.D., Ph.D.)

Immunology and Microbiology II

page 23

- 1:30 p.m.** FAT10: a novel mediator of HIV viral protein r in HIV-associated nephropathy
Alexandra Snyder, Mount Sinai School of Medicine of New York University (Paul E. Klotman, M.D., and Michael J. Ross, M.D.)
- 1:45 p.m.** The 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, West Virginia School of Osteopathic Medicine (Rick M. Fairhurst, M.D., Ph.D.)
- 2:00 p.m.** The C proteins of human parainfluenza virus type 1 (HPIV1) control a broad array of cellular genes, orchestrating a stealth attack
Jim B. Boonyaratanakornkit, University of California, San Francisco, School of Medicine (Brian R. Murphy M.D.)
- 2:15 p.m.** The role of the viral polymerase protein PB1 in the genesis of influenza pandemics
Brett Jagger, Indiana University School of Medicine (Jeffery K. Taubenberger, M.D., Ph.D.)
- 2:30 p.m.** Prevalence of infection with multiple strains of *Mycobacterium tuberculosis* among patients with pulmonary tuberculosis in Kampala, Uganda
Katherine Dickman, University of Pittsburgh School of Medicine (Christopher C. Whalen, M.D.)
- 2:45 p.m.** The lipid mediator and chemokine sphingosine 1-phosphate displays cytokine-like properties in enhancement of Th17 differentiation
Scott Steward-Tharp, University of Iowa College of Dentistry (John J. O'Shea, M.D.)
- 3:00 p.m.** Effect of a novel toll-like receptor 4 (TLR4) antagonist antibody in acute murine colitis
Ryan Ungaro, Mount Sinai School of Medicine of New York University (Maria T. Abreu, M.D., and Lloyd F. Mayer, M.D.)
- 3:15 p.m.** Different HOX gene expression in the ascending versus descending thoracic aorta results in molecular differences and sensitivity to obstructive and aneurismal disease
Sheng-fu Lo, Yale University School of Medicine (George Tellides, M.D., Ph.D.)

SCHEDULE OF PRESENTATIONS

MONDAY
ROOM B

Biomedical Engineering, Biochemistry, and Bioinformatics

page 28

- 9:00 a.m.** Endovascular treatment of aneurysms with a tissue-engineered fibrin biopolymer in a rabbit elastase aneurysm model
Joshua Paul Aronson, Harvard Medical School (Christopher S. Ogilvy, M.D., and Joseph P. Vacanti, M.D.)
- 9:15 a.m.** A novel anti-angiogenic function of A20: implications in diabetic retinopathy
Lynn Y. Choi, New York Medical College (Christiane Ferran, M.D., Ph.D.)
- 9:30 a.m.** Evaluation of nitrite as a putative effector of the ischemic preconditioning cytoprotective program
Shashank S. Sinha, The University of Chicago Pritzker School of Medicine (Mark T. Gladwin, M.D.)
- 9:45 a.m.** Seizure suppression by low-frequency electrical stimulation of the fimbria in combined hippocampus-entorhinal cortex slices in rats
Sheela Toprani, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Dominique Durand, Ph.D., and Imad Najm, M.D.)
- 10:00 a.m.** The physico-chemical and radioprotective properties of synthetic melanins
Andrew D. Schweitzer, Mount Sinai School of Medicine of New York University (Ekaterina Dadachova, Ph.D., and Arturo Casadevall, M.D., Ph.D.)
- 10:15 a.m.** Functional genomics of *Vibrio cholerae*: a systems biology approach to vaccine and drug development
Sanjat Kanjilal, Harvard Medical School (Stephen B. Calderwood, M.D.)

Neuroscience I

page 31

- 1:30 p.m.** Cell-specific transcriptional profiling of medium spiny neurons in Huntington's disease models
Robert Jonathan Fenster, Weill Cornell Medical College (Paul Greengard, Ph.D.)
- 1:45 p.m.** Subcellular localization of Parkin, a Parkinson's disease-related E3-ligase
Derek Paul Narendra, University of Michigan Medical School (Richard Youle, Ph.D.)
- 2:00 p.m.** New interneurons in the mouse neocortex
Jessica S. Choe, Drexel University College of Medicine (Heather A. Cameron, Ph.D.)
- 2:15 p.m.** Vascular endothelial growth factor mediates corneal nerve repair
Charles Q. Yu, University of California, Davis, School of Medicine (Mark I. Rosenblatt, M.D., Ph.D.)
- 2:30 p.m.** Images of chimerism: Schwann cell migration into peripheral nerve allografts
Elizabeth L. Whitlock, Washington University School of Medicine (Susan E. Mackinnon, M.D.)
- 2:45 p.m.** Cerebrospinal fluid-mediated behavior of glioblastoma multiforme: evaluation of invasion, proliferation, and stem cell characteristics
Frank Joseph Attenello, Johns Hopkins University School of Medicine (Alfredo Quiñones-Hinojosa, M.D., and Hongjun Song, Ph.D.)
- 3:00 p.m.** Registration of a NIRS functional time-series dataset in MRI space
Paul Campion, New York University School of Medicine (Eric M. Wassermann, M.D.)

Cancer Biology I

page 35

- 9:00 a.m.** Cisplatin-based chemotherapy enhances the therapeutic potential of antigen-specific immunotherapy
Jorge A. Caballero, Stanford University School of Medicine (James W. Hodge, Ph.D.)
- 9:15 a.m.** The protective role of TRAP1 and Hsp90: stress pathways in cancer
Adam Kern, The University of Chicago Pritzker School of Medicine (Len Neckers, Ph.D.)
- 9:30 a.m.** Glycogen synthase kinase 3 (GSK-3) as a potential target for acute myeloid leukemia (AML) differentiation
Loretta S. Li, Harvard Medical School (Kimberly Stegmaier, M.D.)
- 9:45 a.m.** Targeting CD47 eliminates human acute myeloid leukemia stem cells by disrupting a mechanism of immune evasion
Mark P. Chao, Stanford University School of Medicine (Irving L. Weissman, M.D.)
- 10:00 a.m.** In vivo and in vitro characterization of the role of NF2 in wound healing
Sandy Mong, Harvard Medical School (Andrea McClatchey, Ph.D., and Dennis Orgill, M.D. Ph.D.)
- 10:15 a.m.** Regulation of epithelial genomic instability by stromal fibroblasts in breast carcinogenesis
Cynthia Ann Jimenez, University of California, San Francisco, School of Medicine (Thea Tlsty, Ph.D.)
- 10:30 a.m.** The study of pulmonary metastases using a novel ex vivo lung organ culture assay
M. Ali Khan, University of California, Los Angeles, David Geffen School of Medicine at UCLA (Chand Khanna, D.V.M., Ph.D.)

Cancer Biology II

page 38

- 1:30 p.m.** Xenotropic murine leukemia virus-related virus: a possible role of envelope proteins in chronic inflammation of the prostate
Shoshana Weiner, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Robert H. Silverman, Ph.D., and Eric A. Klein, M.D.)
- 1:45 p.m.** Prognosis in esophageal cancer patients by expression of six cytokine genes
Giang Huong Nguyen, Albany Medical College (Curtis C. Harris, M.D.)
- 2:00 p.m.** The efficacy of selective nuclear factor- κ B inhibitors in cancer cachexia
Ashley Wysong, Duke University School of Medicine (Albert Baldwin, Ph.D., and Marion E. Couch, M.D., Ph.D.)
- 2:15 p.m.** Attenuated transforming growth factor- β signaling promotes nuclear factor- κ B activation in head and neck cancer
Jonah Cohen, The Warren Alpert Medical School of Brown University (Carter Van Waes, M.D., Ph.D.)
- 2:30 p.m.** The role of Yes-associated protein in head and neck cancer
Reza Ehsanian, Stanford University School of Medicine (Carter Van Waes, M.D., Ph.D.)
- 2:45 p.m.** Human papillomavirus type 16 E6-mediated PTPN13 loss synergizes with MAPK signaling downstream of ErbB2 to allow invasive growth in head and neck squamous cell carcinogenesis
Andrew C. Hoover, University of Iowa Roy J. and Lucille A. Carver College of Medicine (John H. Lee, M.D.)

SCHEDULE OF PRESENTATIONS

MONDAY
ATRIUM

Poster Session A, 3:30–4:15 p.m.

Cancer Biology, Vascular Biology, and Biomedical Engineering

page 42

- Poster A1** Development and characterization of CD34-conjugated nanoparticles and a magnetic bone marrow biopsy for evaluating acute leukemia
Jason E. Jaetao, University of New Mexico School of Medicine (Richard S. Larson, M.D., Ph.D.)
- Poster A2** Developmental mimicry in the metastatic niche: modeling leukemic metastasis in vitro and in vivo
Ajay Malhotra, Keck School of Medicine of the University of Southern California (Shahin Rafii, M.D.)
- Poster A3** The role of KiSS-1 in type III TGF- β receptor-mediated metastasis suppression
Michael Sangmin Lee, Duke University School of Medicine (Gerard C. Blobe, M.D., Ph.D.)
- Poster A4** Aberrant JAK2 transcription and miRNA dysregulation in spontaneous STAT1^{-/-} murine mammary adenocarcinoma
Charles Rickert, Washington University School of Medicine (Robert D. Schreiber, Ph.D.)
- Poster A5** Identifying the CA125 binding domain of mesothelin
Osamu Fernando Kaneko, University of California, Los Angeles, David Geffen School of Medicine at UCLA (Ira Pastan, M.D.)
- Poster A6** Identification and characterization of *TMPRSS2/ERG* transcriptional targets in prostate carcinogenesis
Craig P. Giacomini, Stanford University School of Medicine (Jonathan R. Pollack, M.D., Ph.D.)
- Poster A7** Combining targeted antivascular gene therapy with multiple compounds directed against the tumor microenvironment inhibits tumor growth
John S. Quick, New York University School of Medicine (Steven K. Libutti, M.D.)
- Poster A8** Low-field paramagnetic resonance imaging of glycolytic activity and tumor oxygenation following anti-angiogenic therapy in mice
Sonny Batra, Stanford University School of Medicine (James Mitchell, Ph.D.)
- Poster A9** Correlation between Taqman low-density array and oligonucleotide microarrays in measuring multidrug resistance gene expression levels in the NCI-60 cell lines
Josiah N. Orina, Emory University School of Medicine (Michael M. Gottesman, M.D.)
- Poster A10** Raising the bar for genomic prediction: applying statistical methods to ensure that success is not due to chance
Bradford Perez, Duke University School of Medicine (Anil Potti, M.D.)

Poster Session B, 4:15–5:00 p.m.

Immunology, Microbiology, and Molecular Biology

page 47

- Poster B1** Diversity, evolution, and pathogenesis of HIV-1 subtype C: reverse-transcriptase sequence analysis and the selection of nevirapine resistance
Sudeb C. Dalai, Stanford University School of Medicine (David Katzenstein, M.D.)
- Poster B2** Mechanisms of innate immune protection from HIV-1 disease in South Africa
Ambrose Hon Wai Wong, Washington University School of Medicine (Marcus Altfeld, M.D., Ph.D., and William Carr, D.V.M., Ph.D.)
- Poster B3** The role of caspase-7 in restricting *Legionella pneumophila* replication in macrophages
Kyle Viani, University of Michigan Medical School (Gabriel Nuñez, M.D.)
- Poster B4** Evolution of multidrug resistance in *Pseudomonas aeruginosa* clinical isolates
Anvy Nguyen, University of California, San Francisco, School of Medicine (Jeanine P. Wiener-Kronish, M.D., and Susan V. Lynch, Ph.D.)
- Poster B5** Transforming growth factor- β signaling abnormalities in central nervous system inflammatory diseases
Elise M. Meoli, University of Rochester School of Medicine and Dentistry (Steven Jacobson, Ph.D.)
- Poster B6** Effect of mammalian target of rapamycin inhibition on CD4⁺CD25^{high}FOXP3⁺ regulatory T cells compared with conventional CD4⁺ T cells
Elizabeth A. Zambricki, Stanford University School of Medicine (Robert Negrin, M.D.)
- Poster B7** Increasing expression or function of CXCR4, the receptor for SDF-1, enhances hematopoietic stem cell homing and engraftment
Kristin Marie Berg, Creighton University School of Medicine (Harry L. Malech, M.D.)
- Poster B8** Targeting survivin as an antigen for development of a pediatric tumor mouse model
Haven Garber, The Ohio State University College of Medicine (Crystal L. Mackall, M.D.)
- Poster B9** Investigation of the use of myxoma virus as oncolytic therapy for human melanoma
Tanvi Parikh, Weill Cornell Medical College (Liang Deng, M.D., Ph.D., and Stewart Shuman, M.D., Ph.D.)
- Poster B10** Evaluating the function of p53 mutants and prospective cancer treatments in single cells
Irun Bhan, Harvard Medical School (Galit Lahav, Ph.D.)
- Poster B11** Transcriptional profiling of abdominal aortic aneurysms for disease-specific proteins
Mark Hsu, Stanford University School of Medicine (Philip S. Tsao, Ph.D.)
- Poster B12** The role of Bcl-x_L in retinal pigment epithelial cell survival in mouse models of age-related macular degeneration
Ian Carlos Han, Duke University School of Medicine (Glenn J. Jaffe, M.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
ROOM A

Molecular Biology

page 53

- 8:30 a.m.** Aquaporin-2 trafficking: identifying binding partners using mass spectrometry as a hypothesis-generating approach
Nicholas A. Zwang, Harvard Medical School (Mark A. Knepper, M.D., Ph.D.)
- 8:45 a.m.** Nitric oxide stimulates inducible cAMP early repressor (ICER) gene expression in rat pulmonary smooth muscle cells via a Ca²⁺-dependent pathway
Kim Jiramongkolchai, Vanderbilt University School of Medicine (Kenneth D. Bloch, M.D.)
- 9:00 a.m.** Characterization of primary and restenotic atherosclerotic plaque from the superficial femoral artery: differential expression of the TGF- β signaling protein, Smad3
Rachel S. Edlin, New York University School of Medicine (K. Craig Kent, M.D.)
- 9:15 a.m.** Prevention of corneal neovascularization in vivo by transplantation of endostatin-expressing corneal epithelial cells via silk biomatrix
Charles Kim, University of California, Davis, School of Medicine (Mark I. Rosenblatt, M.D., Ph.D.)
- 9:30 a.m.** Melatonin suppresses peripheral *pro-opiomelanocortin* expression: a proposed mechanism of seasonal affective disorder
Joshua Schulman, Harvard Medical School (David E. Fisher, M.D., Ph.D.)
- 9:45 a.m.** Fluorescence-enhanced imaging as an aid to the surgical cytoreduction of peritoneal carcinomatosis of ovarian origin: evaluation in a murine model
Michelle Rae Longmire, University of New Mexico School of Medicine (Peter L. Choyke, M.D.)
- 10:00 a.m.** Autocrine function of fibroblast growth factor 23 in bone mineralization and osteoblast differentiation in vitro
Somi Kim, Harvard School of Dental Medicine (Beate Lanske, Ph.D.)

Neuroscience II

page 57

- 8:30 a.m.** Investigating the effects of Tau aggregates on primary neurons
Amie Yoo-Youn Lee, University of California, San Francisco, School of Medicine (Marc I. Diamond, M.D.)
- 8:45 a.m.** Stability and degradation of the survival of motor neuron protein
Eric C. Muñoz, Oregon Health and Science University School of Medicine (Kenneth H. Fischbeck, M.D.)
- 9:00 a.m.** Subventricular neural progenitor cells differentiate into oligodendrocytes after status epilepticus
Charles Mikell, Columbia University College of Physicians and Surgeons (Guy McKhann, M.D., and James Goldman, M.D., Ph.D.)
- 9:15 a.m.** The functional role of aquaporin-4 in epileptogenesis
Darrin J. Lee, University of California, Irvine, School of Medicine (Devin K. Binder, M.D., Ph.D.)
- 9:30 a.m.** Electrophysiology and functional magnetic resonance imaging during spike-wave seizures in WAG/Rij rats
Damien J. Ellens, Yale University School of Medicine (Hal Blumenfeld, M.D., Ph.D.)

Immunology and Microbiology III

page 59

- 9:45 a.m.** Characterization of early murine cytomegalovirus infection in vivo
Kimberly Hsu, Washington University School of Medicine (Wayne Yokoyama, M.D.)
- 10:00 a.m.** Establishment of an immunosuppressive environment by cutaneous squamous cell carcinomas and its reversal by imiquimod
Susan Jen Huang, Harvard Medical School (Rachel A. Clark, M.D., Ph.D., and Thomas S. Kupper, M.D.)
- 10:15 a.m.** Antigen presentation by lymph node stroma: potential for tolerogenic immunotherapy
Ai-ris Yonekura Collier, Harvard Medical School (Shannon J. Turley, Ph.D.)

SCHEDULE OF PRESENTATIONS

Cancer Biology III

page 61

- 8:30 a.m.** Methods toward understanding cell differentiation and self-renewal in hepatocellular carcinoma
Fei Dong, Case Western Reserve University School of Medicine (Xin Wei Wang, Ph.D.)
- 8:45 a.m.** Combination therapy of radiofrequency ablation and bevacizumab in the treatment of ectopic hepatocellular carcinoma
Ashesh Thaker, University of California, Los Angeles, David Geffen School of Medicine at UCLA (Bradford Wood, M.D.)
- 9:00 a.m.** Hypoxia-inducible factor isotypes in physiology and disease
Krishna Parekh, Duke University School of Medicine (M. Celeste Simon, Ph.D.)
- 9:15 a.m.** Histone deacetylase inhibitors upregulate Notch1 and inhibit growth in pheochromocytoma cells
Joel T. Adler, University of Wisconsin School of Medicine and Public Health (Herbert Chen, M.D.)
- 9:30 a.m.** Inflammation-induced sonic hedgehog and notch pathways: impact on gliomagenesis
Paula McPoland, University of Washington School of Medicine (James M. Olson, M.D., Ph.D.)
- 9:45 a.m.** MicroRNA signatures of differentiating mouse embryonic neural stem cells: implications for glioblastoma multiforme
Chiba Ene, Indiana University School of Medicine (Howard Fine, M.D.)
- 10:00 a.m.** In vitro and in vivo radiosensitization of glioblastoma multiforme by poly (ADP-ribose) polymerase inhibitor, GPI21016
Andrea L. Russo, Dartmouth Medical School (Kevin A. Camphausen, M.D.)

Poster Session C, 5:30–6:15 p.m.

Cell and Developmental Biology, and Genetics

page 65

- Poster C1** Maintenance of bone marrow-derived adult mesenchymal stem cells: the role of interleukin-6
Katie L. Pricola, Stanford University School of Medicine (Rocky S. Tuan, Ph.D.)
- Poster C2** Cell migration and adhesion in natural three-dimensional extracellular matrices
Kirsi Hakkinen, Harvard School of Dental Medicine (Kenneth Yamada, M.D., Ph.D.)
- Poster C3** Characterization of novel β -1-adrenergic receptors with preferential β -arrestin-mediated signaling
Priyesh A. Patel, Duke University School of Medicine (Howard A. Rockman, M.D.)
- Poster C4** p21^{cip1} inhibits NF- κ B-mediated transcriptional activation of RANTES expression in vascular smooth muscle cells
Rohit Gupta, Duke University School of Medicine (Manfred Boehm, M.D.)
- Poster C5** Quantification of pancreatic islet tissue
Victor Sanoe Harrison, University of Hawaii, John A. Burns School of Medicine (David M. Harlan, M.D.)
- Poster C6** Retinol binding protein 4 regulates pancreatic islet β -cell function
Camille Michael Minder, Duke University School of Medicine (Christopher B. Newgard, Ph.D.)
- Poster C7** Role of gangliosides in survival and proliferation of human cutaneous carcinomas
Amrita Arora, The University of Chicago Pritzker School of Medicine (Amy S. Paller, M.D.)
- Poster C8** Evaluation of possible candidate genes involved in ocular-specific development, including optic fissure closure and retinal pigmentation
Amana Akhtar, University of Michigan Medical School (Brian Brooks, M.D., Ph.D.)
- Poster C9** The in vitro effects of a single nucleotide polymorphism on expression of extracellular matrix protein Laminin γ -1, a gene important in the pathogenesis of pelvic organ prolapse
Valerie A. Arboleda, University of California, Los Angeles, David Geffen School of Medicine at UCLA (Eric Vilain, M.D., Ph.D., and Larissa V. Rodríguez, M.D.)
- Poster C10** Ciliary dysfunction as an underlying etiology linking primary ciliary dyskinesia with heterotaxy and complex congenital heart disease
Matthew Swisher, Duke University School of Medicine (Cecilia Lo, Ph.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
CLOISTER

Poster Session D, 6:15–7:00 p.m.

Genetics and Neuroscience

page 70

- Poster D1** Ovarian inclusion formation in fragile X-associated primary ovarian insufficiency
John Joseph DeCaro, Stanford University School of Medicine (Stephanie S. Sherman, Ph.D., and Stephen T. Warren, Ph.D.)
- Poster D2** Mutational analysis of signal transducing gene families in cutaneous malignant melanoma
Lavanya H. Palavalli, University of Missouri-Columbia School of Medicine (Yardena Samuels, Ph.D.)
- Poster D3** Effect of C₁-tetrahydrofolate synthetase variants on nutritional and cellular phenotype
Kelly Cushing, Rush Medical College (Lawrence C. Brody, Ph.D.)
- Poster D4** Novel mutations in *FLNA* are associated with tetralogy of fallot and aortic aneurysm
Nishant Dinesh Patel, Johns Hopkins University School of Medicine (Harry C. Dietz, M.D.)
- Poster D5** Action selection in psychogenic movement disorder: an fMRI study
Christina Ann Brezing, University of Florida College of Medicine (Mark Hallett, M.D.)
- Poster D6** A developmental fMRI study of cognitive flexibility
Julie M. Hall, University of California, San Francisco, School of Medicine (Ellen Leibenluft, M.D.)
- Poster D7** Advanced imaging and intra-arterial thrombolysis in large-vessel strokes
Luis A. Verduzco, Harvard Medical School (Ramon G. Gonzalez, M.D., Ph.D.)
- Poster D8** Hypoxic preconditioning reduces cerebral vasospasm and improves functional outcome following experimental subarachnoid hemorrhage
Eric Milner, Washington University School of Medicine (Gregory Joseph Zipfel, M.D., and David Michael Holtzman, M.D.)
- Poster D9** Netrin-4 enhances angiogenesis and neurological outcome after cerebral ischemia
Stanley Hoang, Stanford University School of Medicine (Gary K. Steinberg, M.D., Ph.D.)
- Poster D10** The role of inflammatory cytokines in altered fetal neural progenitor cell behavior
Andra L. Dingman, Stanford University School of Medicine (Theo D. Palmer, Ph.D.)
- Poster D11** In vivo manipulation of Kv4.2 expression and subsequent electrophysiologic characterization in hippocampal CA1 pyramidal cells
Abigail Rao, Dartmouth Medical School (Dax Hoffman, Ph.D.)
- Poster D12** Melanopsin bistability and its implications on intrinsically photosensitive retinal ganglion cell firing in circadian rhythm entrainment
Kareem Mawad, Washington University School of Medicine (Russell N. Van Gelder, M.D., Ph.D.)

- 9:00 a.m.** Myeloperoxidase-catalyzed carbamylation of low-density lipoprotein and its interaction with scavenger receptor class A type 1: a physiologically relevant mechanism promoting cardiovascular disease
Robert A. Koeth, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Stanley Hazen, M.D., Ph.D.)
- 9:15 a.m.** The role of endoplasmic reticulum stress in age-related macular degeneration
Mrinali Patel, Duke University School of Medicine (Chi-Chao Chan, M.D.)
- 9:30 a.m.** Small-molecule inhibition of Hedgehog signaling in adult mouse bone homeostasis
Joshua A. Gordon, University of California, Los Angeles, David Geffen School of Medicine at UCLA (Yingzi Yang, Ph.D.)
- 9:45 a.m.** BMP-mediated bone regeneration and its interactions with the Wnt pathway
Steve Minear, Stanford University School of Medicine (Jill Helms, D.D.S, Ph.D.)
- 10:00 a.m.** Purification and systemic injection of murine bone marrow stromal cells
Brian Sworder, Boston University School of Medicine (Pamela G. Robey, Ph.D.)
- 10:15 a.m.** Stromal cell-derived factor 1 α (SDF-1 α) expression by intravenously administered mesenchymal stem cells to rats after stroke injury: effect on neuroprotection, neurological outcome, and angiogenesis
Amir Khan Durrani, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Marc S. Penn, M.D., Ph.D.)

SCHEDULE OF PRESENTATIONS

WEDNESDAY
ROOM B

Epidemiology

page 79

- 9:00 a.m.** Alcohol consumption and risk of breast cancer in postmenopausal women: the NIH-AARP diet and health study
Quan Lan Jasmine Lew, The University of Chicago Pritzker School of Medicine (Arthur Schatzkin, M.D., Dr.P.H., and Yikyung Park, Sc.D.)
- 9:15 a.m.** Determinants of seroconversion among HPV-16 and HPV-18-infected women in the enrollment phase of the HPV-16 and -18 vaccine trial in Guanacaste, Costa Rica
Christina Bennett, Indiana University School of Medicine (Allan Hildesheim, Ph.D.)

Genetics

page 80

- 9:30 a.m.** Elucidating the role of variants in insulin-like growth factor 2 mRNA binding protein 2 in the pathogenesis of type 2 diabetes
Jesse J. Hanisch, Creighton University School of Medicine (Francis S. Collins, M.D., Ph.D.)
- 9:45 a.m.** Mechanisms of atrial fibrillation: common polymorphisms in the connexin-40 gene *GJA5*
Robert Wirka, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University (Jonathan D. Smith, Ph.D., and David Van Wagoner, Ph.D.)
- 10:00 a.m.** Genetic susceptibility to murine therapy-related acute myeloid leukemia
Ryan Kevin Funk, Washington University School of Medicine (Timothy A. Graubert, M.D.)
- 10:15 a.m.** An effort to create a factor IX expression vector that allows sustained gene expression through the placement of nucleosome positioning signals
Lia EunHee Gracey, University of Michigan Medical School (Andrew Z. Fire, Ph.D., and Mark A. Kay, M.D., Ph.D.)
- 10:30 a.m.** Analyzing variation in gene regulation in humans: global analysis of NF- κ B binding using Chip-Seq in different individuals
Maya Kasowski, Yale University School of Medicine (Michael Snyder, Ph.D.)

ABSTRACTS OF PRESENTATIONS

MONDAY
ROOM A

9:00 A.M.

Live imaging of natural regulatory T cells during *Leishmania major* infection

DAVID CHOU, University of Pittsburgh School of Medicine

Preceptors: Ronald N. Germain, M.D., Ph.D., and Yasmine Belkaid, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ Regulatory T cells (Tregs) are thought to be important in a myriad of immune responses, yet how they act is poorly understood. Two-photon microscopy allows imaging of cells in a live mouse and is helping to shed light on Treg function. Previous intravital imaging of Tregs has focused on dynamics of transgenic cells within lymph nodes. This project examines the behavior of polyclonal Tregs in the dermis during parasitic leishmania infection.

To observe the behavior of T cells *in vivo*, a variety of cell transfer models involving actin-CFP and Foxp3-EGFP transgenic mice were used in both immune deficient and immune competent settings. For parasite visualization, mice were infected in the ear dermis with RFP expressing leishmania.

Initial work shows leishmania segregates into multiple foci per ear, ranging from tens to hundreds of microns. Teffs and Tregs are markedly enriched around these regions and T cell density drops dramatically more than ~100 microns away. Comparison of Tregs and Teffs early on in the infectious process reveals that Tregs are slow moving and appear to make tight stable contacts with infected macrophages. This is different later in the infection when both cell populations form tight contacts with infected cells. Similar to previously published data, Tregs and Teffs rarely form tight stable contacts *in vivo*.

Based on these observations, we believe that Tregs are not acting on Teffs via direct contact-dependent mechanisms at the site of leishmania infection. Rather, their effect near the lesion is more likely mediated through secreted cytokines and/or contact with antigen-presenting cells. Work is being done to confirm these observations as well as to characterize Treg targets in the skin.



D. CHOU



S. BRAUER

9:15 A.M.

Role of Rab11a and Rab11b GTPases in T cell receptor trafficking

STEVEN BRAUER, Harvard Medical School

Preceptor: Lawrence E. Samelson, M.D., National Cancer Institute, National Institutes of Health

■ The T cell antigen receptor (TCR) mediates antigen recognition and cellular activation by binding to peptides that are presented on the surface of antigen-presenting cells (APCs). The surface expression of the TCR is regulated by dynamic vesicular delivery, internalization, and recycling processes. During activation, these processes accelerate and may preferentially target the TCR to the site of contact with the APC. For many other receptors, Rab GTPases, which couple the unidirectional transport of vesicular cargo to the hydrolysis of GTP, regulate vesicular movement, or trafficking, between intracellular compartments and the plasma membrane. We asked what role, if any, Rabs might play in TCR trafficking.

To identify which members of the Rab family might be involved in TCR trafficking, we transfected various RFP-Rab fusion proteins into Jurkat T cells that stably express the TCR zeta subunit fused to YFP (TCR ζ -YFP). We then used confocal microscopy to visualize RFP-Rab and TCR ζ -YFP trafficking during activation of live cells. Using this approach, we found that RFP-Rab11a colocalizes with TCR ζ -YFP in intracellular puncta that may represent vesicles. To investigate the functional significance of this finding, we applied site-directed mutagenesis to create constitutively GDP-bound (S25N) and GTP-bound (Q70L) RFP-Rab11a mutants. RFP-Rab11aS25N had a more diffuse distribution than wild-type RFP-Rab11a or Rab11aQ70L, and it did not colocalize with TCR ζ -YFP. However, neither mutant produced an appreciable alteration in TCR ζ -YFP trafficking, relative to wild-type RFP-Rab11a.

Our inability to observe an effect of mutant RFP-Rab11a on TCR ζ -YFP trafficking may indicate the absence of a functional relationship between Rab11a and TCR ζ . However, it may also be due to low expression levels of the mutants or to redundancy of function, perhaps with Rab11b. Ongoing experiments use siRNA knockdown of endogenous Rab11a and/or Rab11b to assess what role, if any, these proteins play in TCR trafficking, and more broadly, in immune activation.

9:30 A.M.

Effector and regulatory T lymphocyte populations as determinants of autoimmunity in a model of systemic inflammatory disease**DAVID CHRISTOPHER CARETTO**, University of California, San Francisco, School of Medicine

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

■ The immune system has the remarkable ability to simultaneously recognize and eliminate an enormous diversity of microbial antigens while remaining unresponsive to self-antigens. This ability relies on a balance between populations of CD4 T cell subsets such as the recently described Th17 effector population and protective regulatory T cells (Tregs) to coordinate host responses while avoiding injury to self. Autoimmunity arises when this balance is altered. The immune system orchestrates this finely tuned response through the use of inhibitory receptors such as CTLA-4, a negative regulator of T cell activation and proliferation. Mice lacking CTLA-4 develop lethal multiorgan inflammatory reactions, and polymorphisms in the CTLA-4 gene are associated with numerous human autoimmune diseases. How CTLA-4 works to control immune responses is unclear.

To address CTLA-4's effects on the balance between effectors and regulatory T cells, we transferred a naïve monoclonal population of wild-type and CTLA-4-deficient T cells specific for ovalbumin into lymphopenic mice expressing this protein. After contacting their self-antigen, the activated T cells proliferate into Th17 and Th1 effector populations and create severe inflammatory disease in the mice. This disease resolves at later time points, and resolution correlates with the development of a Treg population. In this model of systemic inflammation, CTLA-4-deficient T cells exhibit increased cycling, greater expansion of responding T cells, and persistence of a Th17 population throughout the course of disease. The percentage of FoxP3⁺ Tregs that do develop is reduced in the absence of CTLA-4 and may be functionally defective.

We conclude that CTLA-4-deficient T cells preferentially develop into a Th17 population at the expense of an adequate functional Treg population that would be necessary for controlling this effector subset. In the absence of a functional Treg subset, the effector population is allowed to remain unchecked, thus contributing to and exacerbating autoimmunity.

9:45 A.M.

Assessing the capacity of bone marrow-derived plasmacytoid and myeloid dendritic cells to promote graft survival via induction of regulatory T cells**ERIC GEHRIE**, Mount Sinai School of Medicine of New York University

Mentor: Jonathan S. Bromberg, M.D., Ph.D., Recanati/Miller Transplantation Institute and Mount Sinai School of Medicine of New York University

■ Immunosuppressive drugs work systemically and cannot be given in large enough doses to completely suppress immunity to a graft without simultaneously obliterating immunity against pathogens. Regulatory T cells (Treg) may hold the key to graft-specific tolerance because of their unique ability to suppress effector T cell functions in peripheral tissues, such as a graft. Our research aims to identify specific subsets of dendritic cells that may induce Treg-mediated tolerance and enhance graft survival.

We have successfully cultured both plasmacytoid (pDC) and myeloid (mDC) dendritic cells from murine bone marrow cells in vitro using recombinant mouse fms-like tyrosine-kinase 3 ligand. Separately, we have generated mDC using recombinant murine granulocyte macrophage colony stimulating factor. The cultured cells have been confirmed to express surface markers consistent with mDC or pDC by flow cytometry. We have also generated secondary cultures of bone marrow-derived mDC and pDC with naïve T cells plus exogenous anti-CD3 ϵ monoclonal antibody, interleukin-2, and transforming growth factor- β . Preliminary results indicate that these secondary cultures induce Treg. Over the coming months, we plan to optimize these secondary cultures and to investigate the tolerogenic potential of bone marrow-derived pDC and mDC in vivo via adoptive transfer of these cells into mice receiving transplants.



D. C. CARETTO



E. GEHRIE

MONDAY
ROOM A

10:00 A.M.

Solution to a long-standing paradox? Disproportionate proliferation of the indirect pathway during acute rejection

HARRAS ZAID, University of California, San Francisco, School of Medicine

Mentors: Sang-Mo Kang, M.D., and Qizhi Tang, Ph.D., University of California, San Francisco

■ In allogeneic transplantation, recipient T cells are activated by graft antigen presented directly on donor antigen-presenting cells (APC) and indirectly by recipient APC. However, the relative contribution of each pathway to alloimmunity is unclear. It is estimated that the precursor frequency of indirect T cells is orders of magnitude lower than that of direct T cells. Thus, the direct pathway has been assumed to be the dominant mediator of acute rejection while the indirect pathway has been implicated in chronic rejection. This paradigm, however, fails to explain the finding that acute rejection is unaltered by elimination of the direct pathway.

To explore the relative contributions of the direct and indirect pathways, we have developed a murine T cell receptor (TCR) transgenic system that can simultaneously track the CD4-direct (CD4-d), CD4-indirect (CD4-i), and CD8-direct (CD8-d) pathways after transplantation. Using this system, we have observed a profoundly greater proliferation and effector phenotype of CD4-i T cells relative to CD4-d and CD8-d T cells after allogeneic skin, heart, or islet transplantation. To rule out an artifact of our TCR transgenic system, we analyzed endogenous, wild-type T cells. In naïve mice, T cells with indirect reactivity were undetectable, while T cells with direct reactivity were abundant. However, 10 days after allogeneic skin or heart transplantation, CD4-i T cells comprised ~10% of the alloreactive CD4+ T cells, and by 60 days comprised ~20%. Furthermore, analysis of graft-infiltrating CD4+ T cells 7 days after heart transplantation showed that CD4-i T cells accounted for approximately 33%.

Thus, indirect cells appear to preferentially expand and accumulate relative to direct cells, allowing comparatively rare CD4-i T cells to comprise a significant proportion of alloreactive CD4+ T cells following transplantation. These findings may have significant implications for rational design of immunosuppressive and tolerance-induction regimens.



H. ZAID



J. M. BARON

10:15 A.M.

Genomic features contributing to chromosomal translocations

JASON M. BARON, Washington University School of Medicine

Mentor: Barry Sleckman, M.D., Ph.D., Washington University School of Medicine

■ Chromosomal translocations are not observed evenly throughout the genome, and many lymphoid tumors contain characteristic translocations. While selection for translocations that promote transformation may contribute to this uneven distribution, we hypothesize that certain genomic locations also have inherent features that make them prone to translocations. To elucidate the influence of inherent genomic features on the distribution of translocations, we generate and track translocations using a cell culture system. This system consists of transformed ATM (ataxia-telangiectasia mutated)-deficient murine pre-B cells with recombination signal sequences (RSSs) inserted at defined genomic locations. The RSSs are cleaved by the products of the recombinase activating genes (RAG) to generate DNA double-stranded breaks. Some of these breaks are resolved as chromosomal translocations, and since the cells are already transformed, primarily factors other than selection should determine the translocation targets.

Using inverse PCR and sequencing, we have catalogued the breakpoints of approximately 150 translocations so far. Our preliminary data suggest a disproportionately large number of translocations to the immunoglobulin light chain κ locus (IgL κ). This locus contains endogenous RSSs that are physiologically cleaved as part of lymphocyte antigen receptor gene assembly. In addition, some of the translocation targets outside of IgL κ appear to contain sequences that bear a resemblance to true RSSs, although the significance of these “cryptic RSSs” remains unclear.

As we assemble a large database of translocations, we hope to confirm the above observations and to note other genomic factors that may contribute to chromosomal translocations, including the higher order nuclear arrangement of chromosomes, the level of transcription activity, and the presence of repetitive elements.

10:30 A.M.

Ex vivo dynamic imaging of retinal microglia using time-lapse confocal microscopy**JUNG EUN LEE**, Duke University School of Medicine

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

■ **Purpose:** Microglia are the resident immune cells of the central nervous system (CNS), including the neural retina. These cells have been implicated in the pathogenesis of neurodegenerative and retinal diseases. This study examines the morphology and behavior of living retinal microglia under resting conditions and in response to focal laser injury.

Methods: Green fluorescent protein (GFP)-labeled microglia in acutely isolated, intact retinal explants from CX3CR1^{+/GFP} transgenic mice were observed using time-lapse confocal imaging. Dynamic changes in overall morphology, microglial process structure, and migratory behavior were recorded and analyzed under resting conditions and after focal laser injury, similar to that used in the treatment of diabetic macular edema.

Results: Resting retinal microglia demonstrate marked structural dynamism in their cellular processes that traverse the extracellular milieu. These process movements have no preferential direction, but are balanced to maintain cellular symmetry and overall arbor size. Resting retinal microglia, while structurally dynamic with a mean process velocity of $5.44 \pm 2.33 \mu\text{m}/\text{min}$ (mean \pm SD, $n=367$ processes from 37 cells in three mice), do not exhibit cellular migration under resting conditions. After focal laser injury, the processes increase rate of movement significantly and acquire a marked tropism toward the center of injury. Retinal microglia postinjury develop polarized cellular morphologies that are oriented toward laser injury and acquire a migratory phenotype that enables them to translocate through retinal tissue.

Conclusions: Retinal microglia, like other CNS microglia, exhibit marked structural motility in their processes that are well adapted to a constant and rapid sampling of their environment. These dynamic features of retinal microglia behavior under resting conditions suggest functions of surveillance and intercellular communication. This finding may have implications for the endogenous role of microglia in the retina and suggest a mechanism for therapeutic aspects of focal laser photocoagulation.

1:30 P.M.

FAT10: a novel mediator of HIV viral protein r in HIV-associated nephropathy**ALEXANDRA SNYDER**, Mount Sinai School of Medicine of New York University

Mentors: Paul E. Klotman, M.D., and Michael J. Ross, M.D., Mount Sinai School of Medicine of New York University

■ HIV-associated nephropathy (HIVAN) is a leading cause of end-stage renal disease (ESRD), primarily in patients of African descent and is characterized by glomerulosclerosis and tubulointerstitial disease. Recent studies, including those from our own laboratory, suggest a central role for *vpr* in HIVAN pathogenesis. In human renal tubular epithelial cells (RTECs) *vpr* causes apoptosis, arrest of the cell cycle at the G2/M phase, and hyperdiploidy. *FAT10* is the most robustly upregulated gene upon HIV infection of RTEC both in vivo and in vitro. In the current studies, we therefore sought to determine the mechanisms by which the ubiquitin-like protein FAT10 mediates proapoptotic and cell-cycle dysregulation induced by HIV-1 viral protein r (Vpr) in renal tubular epithelial cells. Using real-time PCR, we found that infection of RTECs with lentiviral vectors expressing *vpr* leads to threefold upregulation of *FAT10* at 5 days postinfection, as compared to fivefold upregulation upon infection with HIV. Proximal tubule cells from a *FAT10*^{-/-} mouse showed a small increase in hyperdiploidy but were resistant to Vpr-induced apoptosis and G2 arrest. Restoration of *FAT10* expression in *FAT10*^{-/-} cells using FAT10-expressing lentivirus restored Vpr-induced apoptosis and hyperdiploidy. We then performed immunocytochemistry and immunoprecipitation to determine if there is physical interaction between Vpr and FAT10. We found that Vpr and FAT10 colocalized in clumped mitochondria after cotransfection of 293HEK cells with GFP-labeled Vpr and Flag-tagged FAT10 and staining with Mitotracker CMXRos. Moreover, Vpr-infected tubule cells showed a decreased proportion of actively oxidizing mitochondria as determined by mitochondrial staining with CMXRos. We then performed immunoprecipitation using HEK293T cells after cotransfection with xpress-tagged *FAT10* and Flag-tagged *vpr* vectors. We found that xpress-FAT10 coimmunoprecipitated with flag-Vpr. These studies indicate that FAT10 plays a role in the apoptotic function of Vpr, possibly by direct interaction and by facilitating mitochondrial toxicity.

MONDAY
ROOM A

J. E. LEE



A. SNYDER

MONDAY
ROOM A

1:45 P.M.

The 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, 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 is a parasitic disease transmitted by the protozoan *Plasmodium falciparum*. Each year, malaria afflicts more than 300 million people worldwide and kills more than 1 million children in Africa. Genetic polymorphisms for sickle hemoglobin (HbS), HbC, and glucose-6-phosphate dehydrogenase (G6PD) deficiency are common in Africa and have been associated with protection against severe malaria. These polymorphisms accelerate the oxidative denaturation of hemoglobin, resulting in erythrocyte membrane damage and antioxidant depletion. We hypothesize that oxidative damage to erythrocyte membranes interferes with the display and function of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), the parasite's main virulence factor on the surface of infected erythrocytes. PfEMP-1 is localized within cell surface protrusions, called knobs, which mediate the adherence of parasitized erythrocytes to host microvessels and cause severe disease. Our goal is to investigate the role of erythrocyte oxidation on PfEMP-1 display, knob morphology, and adherence of parasitized erythrocytes to human microvascular endothelial cells (HMVECs).

Erythrocytes containing immature-stage parasites will be pulsed for several hours with oxidant compounds, including diethyl maleate, tert-butylhydroperoxide, or the antimalarial drug artemisinin. Oxidative damage will be assessed through biochemical detection of erythrocyte glutathione and hemichrome levels (oxidatively denatured hemoglobin molecules). After 24 hours of culture, mature-stage parasites expressing PfEMP-1 will be viewed by atomic force microscopy (AFM) to determine knob morphology. Surface PfEMP-1 expression will be quantified by flow cytometry using a PfEMP-1-specific antiserum. Adherence of infected erythrocytes to HMVECs will be assessed by an in vitro binding assay.

Any findings of abnormal knob morphologies, reduced surface PfEMP-1 expression, or impaired adherence to HMVECs in our experimental system will be confirmed using naturally circulating *P. falciparum* isolates and polymorphic erythrocytes obtained directly from patients with malaria.



S. BEAUDRY



J. B. BOONYARATANAKORNKIT

2:00 P.M.

The C proteins of human parainfluenza virus type 1 (HPIV1) control a broad array of cellular genes, orchestrating a stealth attack

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

Preceptor: Brian R. Murphy M.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

■ HPIV1 is an important respiratory pathogen in children and the most common cause of viral croup. We compared whole genome microarray-based expression profiles in A549 human respiratory epithelial cells at 6, 12, 24, and 48 h following infection with wt HPIV1 or treatment with interferon (IFN) β . During wt infection, we found 370 differentially expressed transcripts that could be grouped, based on kinetics of expression, into four IFN-dependent clusters and one IFN-independent cluster. Two hundred and fifty transcripts that are induced by both infection or IFN are mainly involved in IFN, NF- κ B, TLR, and chemokine signaling, as well as in antigen presentation and ubiquitination. Conversely, the 120 transcripts that are induced by infection but not IFN are involved in IL6, NF- κ B, death receptor, and apoptosis pathways. HPIV1 encodes four accessory C proteins that inhibit induction of and signaling by type 1 IFN. To study the impact of the C proteins on the host response, we determined expression profiles in A549 cells infected with 1) HPIV1-P(C-), an attenuated, IFN-inducing mutant virus that does not express any of the four C proteins, or 2) HPIV1-C^{F170S}, a less attenuated, but still IFN-inducing mutant that contains a single point mutation in C. The P(C-) mutant also induces apoptosis, whereas the C^{F170S} mutant and wt HPIV1 do not. Infection of A549 cells with either C mutant induced a complex cellular response with 2,612 differentially expressed genes of which only 223 were IFN inducible. The cellular response to C^{F170S} and P(C-) was almost identical, although the P(C-) mutant was more attenuated and induced apoptosis. Two important observations emerge: 1) expression of the C proteins by wt virus alters the expression of over 2,200 host genes and 2) the differences in apoptosis and attenuation phenotypes of the two C mutant viruses are not due to a difference in their expression profiles.

2:15 P.M.

The role of the viral polymerase protein PB1 in the genesis of influenza pandemics**BRETT JAGGER**, Indiana University School of Medicine

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

■ The influenza A virus (IAV) periodically causes worldwide pandemics in humans, resulting in considerable morbidity and mortality. The virus has a stable natural reservoir in migratory waterfowl; reassortment of genome segments between human and avian viruses can yield progeny that are antigenically novel and therefore able to spread rapidly in humans. However, genetic analyses of previous pandemic viruses have shown that in addition to the surface antigens hemagglutinin and neuraminidase, the viral polymerase basic protein-1 (PB1) has also been avian-derived in each of the last three pandemics. Further, in contrast to the evolutionary stasis observed in avian hosts, human IAV PB1s accumulate coding mutations that deviate from the avian consensus. These observations, paired with PB1's central role in IAV polymerase function, suggest a role for PB1 in viral fitness and the evolution of pandemic influenza viruses.

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

Avian-derived 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. Further investigation of this hypothesis via reverse genetics experiments and sequence analysis will improve our understanding of the genesis of influenza pandemics.

2:30 P.M.

Prevalence of infection with multiple strains of *Mycobacterium tuberculosis* among patients with pulmonary tuberculosis in Kampala, Uganda**KATHERINE DICKMAN**, University of Pittsburgh School of Medicine

Mentor: Christopher C. Whalen, M.D., Case Western Reserve University School of Medicine

■ Active tuberculosis disease is generally thought to be caused by infection with a single strain, though a number of molecular studies have demonstrated simultaneous infection with multiple strains of *Mycobacterium tuberculosis*. The extent to which patients in an area have multiple infections has important implications for the interpretation of molecular testing for transmission dynamics, recurrent disease, and drug resistance. A molecular typing method using mycobacterial interspersed repetitive unit (MIRU)-variable-number tandem repeats (VNTR) analysis has been used to detect mixed clonal variants in tuberculosis cases. We are using MIRU-VNTR analysis to determine the prevalence of multiple infections in the high transmission setting of Kampala, Uganda. We are also following patients throughout treatment to describe strain persistence and to determine whether infection with multiple strains has clinical impact on these patients.

To date, we have enrolled 50 patients, 31 of whom reside in households in which we have cultures from both index and contact cases. The median age of these subjects is 24 years (range 6 months to 46 years), and 50% are male. Four (8%) of these subjects are HIV-infected. MIRU-VNTR analysis is ongoing. Thus far, we have collected 91 baseline samples. Three patients have had positive cultures after two months of treatment, and we have collected six samples in total for this time point. We will use the typing results to determine the prevalence of multiple infections among these patients, as well as to describe culture conversion rates throughout treatment and transmission dynamics among household contacts.



B. JAGGER



K. DICKMAN

MONDAY
ROOM A

2:45 P.M.

The lipid mediator and chemokine sphingosine 1-phosphate displays cytokine-like properties in enhancement of Th17 differentiation

SCOTT STEWARD-THARP, University of Iowa College of Dentistry

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

■ Sphingosine 1-phosphate (S1P) is a natural lipid mediator in blood and lymph that controls lymphoid traffic and tissue migration of T cells through signals from the type 1 S1PR (S1P₁). Recently, the effects of the S1P-S1P₁ axis on nonmigration functions of T cells have received increasing interest. Studies using transgenic mice that selectively overexpress S1P₁ on their T cells have linked the S1P-S1P₁ axis to IL-4, FoxP3, and IL-17 upregulation in CD4 T cells. These studies used an artificial system, showed conflicting results, and failed to distinguish whether S1P affected naïve or memory CD4 T cells preferentially. We have thus investigated the role that S1P plays in both naïve and memory CD4 T cell differentiation.

Using both αCD3/CD28 and antigen-pulsed APC stimulation, we separately cultured naïve and memory CD4 T cells in the presence or absence of S1P under Th1, Th2, Th17, and iTreg producing conditions. Most significantly, we observed that S1P enhanced differentiation to the Th17 lineage in an IL-23-like manner, acting on both the naïve and the memory cell population. We have additionally shown that S1P and IL-23 do not act synergistically in enhancing IL-17 expression. Interestingly, blocking the IL-21 autocrine positive feedback loop in Th17 differentiation ameliorated S1P's effects. Using S1P₁ knockout mice, we have verified that S1P's enhancement of Th17 differentiation takes place through the S1P-S1P₁ axis. Germline knockout of S1P lyase, the enzyme that permanently breaks down S1P in mice resulted in a severe autoinflammatory phenotype with death occurring by six weeks of age. The role of the Th17 population, which is highly implicated in several autoimmune disorders, in these mice is currently under investigation.

S1P-S1P₁ signaling enhances Th17 differentiation in CD4 T cell populations in vitro. This pathway could serve a physiologically protective role since high S1P concentrations have been associated with local areas of inflammation.



S. STEWARD-THARP



R. UNGARO

3:00 P.M.

Effect of a novel toll-like receptor 4 (TLR4) antagonist antibody in acute murine colitis

RYAN UNGARO, Mount Sinai School of Medicine of New York University

Mentors: Maria T. Abreu, M.D., University of Miami Miller School of Medicine, and Lloyd F. Mayer, M.D., Mount Sinai School of Medicine of New York University

■ Dysregulated innate immune responses to commensal bacteria contribute to the development of inflammatory bowel disease (IBD). TLR4, a pathogen recognition receptor and key initiator of innate immunity, is overexpressed in the intestinal mucosa of IBD patients. Reducing TLR4 signaling has the potential to alleviate uncontrolled inflammation. The aim of this study is to examine the effect of a novel TLR4 antagonist in a murine model of IBD.

Colitis was induced in mice with 7 days of 2.5% dextran sodium sulfate (DSS) treatment. An antibody (Ab) against the TLR4/MD2 complex was administered intraperitoneally. Control mice received an isotype-matched Ab. Severity of colitis was assessed by disease activity index (DAI) and histology. The effect of the Ab on the inflammatory infiltrate was determined by counting the number of lamina propria dendritic cells (DCs) and macrophages using magnetic sorting and immunohistochemistry. Mucosal expression of innate immunity mediators MIP-2, CX₃CL1, CCL2, and COX-2 and proinflammatory cytokines TNF-α and IL-6 was analyzed by real-time PCR and ELISA.

TLR4 Ab treatment at the beginning of DSS delayed the development of colitis with lower DAI scores the first 2 days. Ab-treated mice had less inflammation at day 7 of DSS with reduced mucosal expression of CCL2, TNF-α, IL-6, and Cox-2 as well as decreased macrophage infiltrate. When TLR4 Ab was administered at the end of DSS it rapidly (within 24 hours) reduced the number of lamina propria DCs compared to controls. Our results demonstrate that TLR4 is an important mediator of innate immune cell recruitment and cytokine production in response to mucosal injury. Since innate immune cells are important in the induction and perpetuation of mucosal inflammation, modulation of TLR4 signaling may alter the course of IBD.

3:15 P.M.

Different HOX gene expression in the ascending versus descending thoracic aorta results in molecular differences and sensitivity to obstructive and aneurismal disease

SHENG-FU LO, Yale University School of Medicine

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

■ Vascular diseases such as atherosclerosis or aneurismal disease selectively affect different parts of the arterial system. Despite this heterogeneous pattern of disease within the arterial system, the contribution of different smooth muscle cell phenotypes to this pattern has not been well studied. Here, we focus on the role of differential HOX gene expression in the ascending versus descending thoracic aorta in mice. A HOX code of the vasculature was described by sampling internal carotid, common carotid, ascending thoracic aorta, descending thoracic aorta, abdominal aorta, and common femoral arteries from mice and studying their HOX expression pattern by RT-PCR. The

regional influence of the surrounding tissue was studied by transplanting ascending and descending thoracic aorta into the abdominal aorta. Persistence of these epigenetic changes was also studied by culturing smooth muscle cells from the ascending and descending thoracic aorta. Lineage tracing was performed by using Cre-lox models involving Hoxb7-cre and Wnt1-cre mice bred to reporter strains using LacZ and different fluorescent proteins. Candidate HOX downstream targets were identified by human microarrays and confirmed by expression studies in mice. The role of HOX genes and their candidate downstream targets was also investigated in mouse models of obstructive and aneurismal disease, using ApoE^{-/-} mice and fibrillin mutant mice, respectively. The role of TGF- β and IFN- γ , characteristic of these disease processes, was studied by treating aortic segments in organ culture. Our preliminary results indicate that the cephalad to caudad progression of the HOX paralogues from 1 to 13 is preserved in the vasculature, with notable differences between the ascending and descending aorta. The results of our ongoing experiments will be discussed.



S. LO

MONDAY
ROOM B

9:00 A.M.

Endovascular treatment of aneurysms with a tissue-engineered fibrin biopolymer in a rabbit elastase aneurysm model

JOSHUA PAUL ARONSON, Harvard Medical School

Mentors: Christopher S. Ogilvy, M.D., and Joseph P. Vacanti, M.D., Harvard Medical School and Massachusetts General Hospital



J. P. ARONSON

■ Intracranial aneurysms are currently treated by surgical clipping or endovascular coil embolization. However, coiling is associated with a relatively high rate of recanalization due to lack of re-endothelialization across the aneurysm ostium. We attempt a tissue-engineering approach to promote endothelialization by seeding endothelial progenitor cells (EPCs) within bioabsorbable fibrin matrix and delivering the construct endovascularly into the aneurysm.

Model aneurysms were created in New Zealand white rabbits using the elastase aneurysm model. Pancreatic elastase was infused into a trapped segment of the right common carotid artery at its bifurcation from the brachiocephalic artery. The arterial segment was then ligated distally and flow restored proximally. At aneurysm creation, EPCs were isolated from the mononuclear fraction of peripheral blood and cultured in vitro for 10 days. Rabbits were untreated or treated endovascularly via right femoral artery catheterization with either fibrin matrix alone or fibrin plus autologous EPCs. Degree of aneurysm occlusion was determined by angiography. Rabbits were sacrificed up to 18 weeks posttreatment. Aneurysms were resected and extent of endothelial growth at the aneurysm neck was evaluated by expression of platelet/endothelial cell adhesion molecule-1 (PECAM-1), an endothelial cell marker.

Aneurysms left untreated remained patent throughout the study period. Aneurysms treated with fibrin alone recanalized within 4 weeks post-treatment. Histology reveals few PECAM-1-positive cells at the aneurysm neck. Aneurysms treated with fibrin and EPCs remained occluded throughout the 16-week follow-up period. Histology demonstrated formation of a PECAM-1-positive cell layer across the aneurysm ostium.

Fibrin biopolymer can be delivered endovascularly to an experimentally created aneurysm, and the EPC-seeded fibrin construct can induce formation of a neointima across the ostium. A tissue-engineering approach addresses the lack of endothelialization that limits the long-term success of coil embolization. Future experiments will evaluate other biopolymers for ease of endovascular delivery and suitability as a matrix for endothelial growth.



L. Y. CHOI

9:15 A.M.

A novel anti-angiogenic function of A20: implications in diabetic retinopathy

LYNN Y. CHOI, New York Medical College

Mentor: Christiane Ferran, M.D., Ph.D., Beth Israel Deaconess Medical Center and Harvard Medical School

■ We sought to determine whether the cytoprotective and NF- κ B inhibitor gene A20 affects angiogenesis and VEGF-mediated signaling, and if so, could it impact the development and progression of diabetic retinopathy (DR). Abnormal retinal angiogenesis and increased susceptibility of endothelial cells (ECs) to apoptosis are the hallmarks of DR. Ischemia-related increase of VEGF is a major pathogenic effector of abnormal angiogenesis in retinas of diabetic patients. VEGF phosphorylates PKC β II and ERK1/2, leading to increased EC migration and proliferation, and activates the pro-survival factor Akt, which is negatively affected by high glucose.

We determined that overexpression of A20 in human retinal EC drastically inhibited tube formation, used as a read-out for angiogenesis. This was not reverted by the addition of VEGF or by culturing the ECs in high glucose. Given that A20 inhibition of tube formation was refractory to VEGF, we evaluated its effect upon downstream effectors of VEGF signaling. Expression of A20 in ECs blocked VEGF-mediated angiogenic signals, including phosphorylation of PKC β II and ERK1/2, while promoting phosphorylation of the survival factor Akt. These results translated into protection of mice pups from oxygen-induced proliferative retinopathy of prematurity (OIR), an accepted model of DR. A20 gene transfer to mouse pups' retina protected from OIR by inhibiting PKC β II and ERK1/2 phosphorylation and enhancing Akt activation. Angiograms of A20-treated eyes were normal in contrast to control eyes that showed severe vascular leaks and peripheral neovascularization.

These data demonstrate a novel anti-angiogenic function of A20 in ECs via selective blockade of the proliferative and migratory signals of VEGF to ECs, while promoting the survival signal mediated by this growth factor through increased Akt phosphorylation. We propose that A20-based therapies may be well suited for the prevention/treatment of DR.

9:30 A.M.

Evaluation of nitrite as a putative effector of the ischemic preconditioning cytoprotective program

SHASHANK S. SINHA, The University of Chicago Pritzker School of Medicine

Preceptor: Mark T. Gladwin, M.D., National Heart, Lung, and Blood Institute, National Institutes of Health

■ Recent studies have demonstrated that physiological concentrations of nitrite mediate potent cytoprotection after ischemia-reperfusion (I/R) in the heart, liver, brain, and kidney. Interestingly, nitrite-dependent cytoprotection bears many similarities to cardioprotection mediated by ischemic preconditioning (IPC), whereby several, brief cycles of sublethal ischemia followed by reperfusion confer protection against a subsequently prolonged ischemic episode. For example, similarly to IPC, nitrite demonstrates two windows of cytoprotection: acute (nitrite is administered immediately prior to reperfusion) and delayed (nitrite is given 24 hours before the ischemic episode). Mechanistically, both IPC and nitrite have been shown to alter mitochondrial function and both treatments result in post-translational S-nitrosation of mitochondrial complex I. Importantly, IPC increases myocardial tissue nitrite levels in both windows.

Hence, we hypothesized that nitrite is an effector of IPC and employed a rat Langendorff isolated perfused heart model of I/R to characterize both IPC- and nitrite-mediated cytoprotection. We measured left ventricular developed pressure (LVDP, the difference between left ventricular systolic pressure and end-diastolic pressure) using a fluid-filled latex balloon. We compared functional recovery of the heart after 20 minutes of global no-flow normothermic (37°C) ischemia followed by 40 minutes of reperfusion after no treatment or treatment with nitrite (10 μM) or IPC. Functional recovery was expressed as the postischemic LVDP as a percentage of preischemic levels.

Both IPC- and nitrite-treated hearts achieved greater functional recovery and reduced time to contracture as compared to control hearts. Low concentrations of nitrite protected hearts from I/R injury to a similar extent as IPC. IPC increased myocardial tissue nitrite levels to a similar extent as low-dose nitrite treatment, and both treatments inhibited mitochondrial complex I, to similar extents, immediately after reperfusion. These data demonstrate similar mechanisms and extents of cytoprotection between IPC and nitrite, providing evidence that nitrite may be an endogenous effector of the IPC cytoprotective program.

9:45 A.M.

Seizure suppression by low-frequency electrical stimulation of the fimbria in combined hippocampus-entorhinal cortex slices in rats

SHEELA TOPRANI, 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 critical consideration for this treatment is selecting a location for stimulation delivery that can effectively diminish seizure activity in the hippocampus, which is heavily implicated in MTLE. The objective of this study is to evaluate the efficacy of LFS of the fimbria, a major efferent and afferent pathway of the hippocampus, in suppressing hippocampal seizures. Spontaneous epileptiform activity was chemically induced in 650-μm-thick transverse rat brain slices using 4-aminopyridine (4-AP) and developed until seizures occurred at a predictable frequency. Field responses were measured in CA3 and CA1 hippocampal subfields. Electrical stimulation (1 Hz) was applied to the fimbria for a time frame of at least five times the seizure frequency. Seizure activity before, during, and after LFS is compared.

In this study, seizures were defined as high amplitude, high frequency spiking period(s) of greater than 10 Hz activity lasting longer than 5 seconds. Percentage of seizure time during LFS of the fimbria was significantly suppressed (0.86%±1.8%) compared to that preceding stimulation (28.2%±13%) (paired t (9)=6.2518, p=0.0001). The percentage of seizure time following the termination of LFS (28.68%±10.3%) was not significantly different from that prior to its onset (paired t (9)=0.0682, p=0.9471). Seizure suppression averaged 89.63%, with 100% suppression in 8 out of 10 slices.

These data demonstrate that LFS of the fimbria effectively suppresses hippocampal seizures in this 4-AP in vitro model. Furthermore, seizure activity recovers after stimulation is removed. This suggests that the fimbria may be an effective location for LFS to alleviate MTLE.



S. S. SINHA



S. TOPRANI

MONDAY
ROOM B

10:00 A.M.

The physico-chemical and radioprotective properties of synthetic melanins

ANDREW D. SCHWEITZER, Mount Sinai School of Medicine of New York University

Mentors: Ekaterina Dadachova, Ph.D., and Arturo Casadevall, M.D., Ph.D., Albert Einstein College of Medicine of Yeshiva University

■ Melanin, a high-molecular-weight pigment that is ubiquitous in nature, is known to protect the skin against the effects of ultraviolet radiation, and melanized microorganisms have a survival advantage in the environment that includes protection against ionizing radiation. Scavenging of the highly destructive free radicals generated by the radiolysis of water has been suggested as a mechanism of radioprotection. This project aims to explore melanin's potential as a radioprotector. We are the first to propose that efficient Compton scattering of incident photons by electron-rich oligomers in the pigment, and trapping of generated recoil electrons and photoelectrons by stable radicals, also contributes to radioprotection. We hypothesize that melanin's radioprotective properties depend on its chemical composition, which can be varied by using different precursors and oxidation methods. Extensive physico-chemical characterization and measurement of surrogate indicators of radioprotection were performed for five melanins synthesized for this project.

Elemental analysis and high-performance liquid chromatography (HPLC) revealed that changing the precursor(s) or oxidation method resulted in chemically distinct melanins composed of different oligomer units, with L-DOPA/L-Cys and 5-S-cysteinyl-DOPA melanins having substantial sulfur composition (22 and 10%, respectively). All melanins studied displayed strong electron paramagnetic resonance, but sulfur-containing melanins had more complex spectra and more stable free radicals. None of the melanins displayed a change in radical quality/quantity after high-dose ^{137}Cs irradiation, demonstrating a high degree of stability. In physical shielding experiments, all tyrosinase-oxidized melanins demonstrated better shielding against irradiation (40–320 kVp) than controls (charcoal and water). 5-S-Cysteinyl-DOPA melanin had the best overall shielding properties.

These physico-chemical data suggest that tyrosinase-oxidized melanins from sulfur-containing precursors are most likely to be radioprotective due to effective free-radical scavenging and physical shielding. Radioprotective properties of these melanins are currently being evaluated using a clonogenic assay of mammalian cells *in vitro*, and will subsequently be evaluated in mice.



A. D. SCHWEITZER



S. KANJILAL

10:15 A.M.

Functional genomics of *Vibrio cholerae*: a systems biology approach to vaccine and drug development

SANJAT KANJILAL, Harvard Medical School

Mentor: Stephen B. Calderwood, M.D., Harvard Medical School

■ *Vibrio cholerae* cause a severe, dehydrating, and occasionally fatal diarrhea in humans. There are an estimated 5 million cases worldwide of cholera each year, with more than 100,000 deaths. The complex sequence of events leading to expression of the essential virulence factors, including cholera toxin, are controlled by the ToxR virulence gene network (ToxR regulon), the primary genetic mechanism responsible for pathogenesis in humans. However, besides the master regulator *toxR* and the primary downstream effector *toxT*, little is known about the identities and interactions of many of the intermediate genes that form the remainder of the network.

We compared the gene expression profile between *V. cholerae* grown in cholera toxin-inducing media and noninducing media by using time-series oligonucleotide microarrays. Thirteen equally spaced time points were taken in each media from the beginning of log phase of growth until stationary phase. Members of the ToxR regulon were ascertained by identifying differentially expressed genes between toxin-inducing and noninducing conditions at early, middle, and late time points. A proposed network structure was constructed using a reverse-engineering algorithm suited to time-series data and will be validated in future experiments using gene knockout experiments to compare *in silico* predictions of network behavior to *in vitro* observations.

Knowledge about the topology of the ToxR regulon will aid in understanding the mechanisms for generating long-lasting immunity, facilitate the development of pharmaceutical therapies that may avoid bacterial resistance, and provide insight into the behavior of biological networks.

1:30 P.M.

Cell-specific transcriptional profiling of medium spiny neurons in Huntington's disease models**ROBERT JONATHAN FENSTER**, Weill Cornell Medical College

Mentor: Paul Greengard, Ph.D., The Rockefeller University

■ Huntington's disease (HD) is a devastating genetic neurodegenerative disorder that presents in mid-life with personality changes and uncontrollable movements, and later leads to hypokinesia, dementia, and death. An autosomal dominant disease, HD is caused by an expanded triplet repeat in the *huntingtin* gene. Intriguingly, despite near ubiquitous expression of *huntingtin* in all neurons, the cell death in HD is highly specific, with medium spiny neurons (MSNs) of the striatum being most vulnerable. Pathological studies of postmortem tissue have shown that within the striatum, MSNs expressing dopamine 2 receptors (Drd2) die before MSNs that express dopamine 1 receptors (Drd1a).

Understanding this selective vulnerability offers a promising therapeutic opportunity, because interventions can be targeted to the early, deleterious events caused by the *huntingtin* mutation. Although there are many transgenic mouse models of HD, it has been impossible to study large-scale gene expression changes in only the vulnerable Drd2-expressing neurons, because the MSNs of the striatum are anatomically intermixed and morphologically indistinguishable.

To address this problem, we are using a novel technology that allows the isolation of mRNAs from genetically defined cell populations. The isolated mRNAs can then be analyzed by microarray. We are performing these experiments in three different HD mouse models, corresponding to different severities of HD, and we are comparing gene expression profiles in vulnerable and nonvulnerable MSNs. These cell-specific studies may identify molecular targets for therapies directed against initiating events in HD.

1:45 P.M.

Subcellular localization of Parkin, a Parkinson's disease-related E3-ligase**DEREK PAUL NARENDRA**, University of Michigan Medical School

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

■ *Parkin* loss-of-function mutations account for up to 50% of early-onset autosomal recessive Parkinson's disease. Although in neural tissue Parkin is found predominately in the cytosol, recent genetic evidence suggests that Parkin may regulate mitochondrial morphology and function. Mitochondria in *Parkin* null *Drosophila melanogaster*, for instance, exhibit abnormal morphology, particularly at stages of spermatogenesis that require exquisite control of mitochondrial transport and fusion. Additionally, in *Drosophila melanogaster* lacking the mitochondrial kinase Pink1, Parkin overexpression rescues the mutant phenotype, including abnormalities observed in mitochondrial morphology. These findings have generated new interest in the subcellular localization of Parkin, and one group recently reported that Parkin is associated with mitochondria in established cell lines. We attempted to replicate these findings in HeLa cells and mouse embryonic fibroblasts (MEFs). Cells were transiently transfected with a YFP-Parkin fusion construct or stained with a monoclonal antibody against Parkin. Parkin subcellular localization was assessed by confocal microscopy.

In both HeLa cells and MEF cells, Parkin was found predominately in the cytosol.

The discovery of new mitochondrial proteins coded by genes linked to familial Parkinson's disease, most notably *Pink1* and *DJ-1*, has put a new focus on mitochondrial dysfunction in the pathogenesis of familial and idiopathic Parkinson's disease. A recent report suggests that Parkin is associated with mitochondria in established cell lines. We were unable to confirm these data. Instead, we found that Parkin is predominately in the cytosol under basal conditions, consistent with earlier findings in neural tissue. Our data suggest that Parkin does not interact with mitochondrial proteins under these conditions.

MONDAY
ROOM B

R. J. FENSTER



D. P. NARENDRA

MONDAY
ROOM B

2:00 P.M.

New interneurons in the mouse neocortex

JESSICA S. CHOE, Drexel University College of Medicine

Mentor: Heather A. Cameron, Ph.D., National Institute of Mental Health, National Institutes of Health



J. S. CHOE

■ Although it is widely accepted that neurogenesis occurs in discrete areas of the adult brain—the dentate gyrus of the hippocampus and the subventricular zone (SVZ)—ongoing neurogenesis in the adult mammalian neocortex is still a topic of debate. Evidence of cortical neurogenesis has previously been found in primates (Gould et al., *Science*, 1999) and rats (Dayer et al., *J Cell Biol*, 2005). Studies in rats revealed newly born GABAergic neurons in the adult neocortex; these cells expressed markers characteristic of a particular class of GABAergic interneurons. In order to elucidate the morphology and confirm the identity of these new neurons, we studied neurogenesis in glutamic acid decarboxylase 65-green fluorescent protein (GAD65-GFP) mice. Twenty-three mice were given a 12-day course of the S-phase marker bromodeoxyuridine (BrdU) and sacrificed six weeks later. Examination of the brains revealed a number of GFP+/BrdU+ cells, the appearance of which seemed consistent with the class of GABAergic interneurons previously described in rats. However, it remains to be elucidated whether these neurons were produced in the subventricular zone and/or the associated rostral migratory stream, as they were in close proximity to, yet not obviously admixed with, the migrating chain of cells, or if they represented a separate population of newly born neurons in the adult brain.



C. Q. YU

2:15 P.M.

Vascular endothelial growth factor mediates corneal nerve repair

CHARLES Q. YU, University of California, Davis, School of Medicine

Mentor: Mark I. Rosenblatt, M.D., Ph.D., Weill Cornell Medical College

■ The cornea is among the most densely innervated tissues of the human body, containing primarily sensory innervation. Corneal nerves are of great importance due to their role in protecting the cornea from irritants as well as their trophic properties, which are necessary to maintain a healthy ocular surface. We examined the vascular endothelial growth factor (VEGF) dependence of corneal nerve regeneration after superficial injury. Trigeminal neurons collected from mice were dissociated and cultured. Cells were grown in neurobasal media with either 250 ng/mL VEGF-specific neutralizing antibody (bevacizumab) or 250 ng/mL nonspecific mouse immunoglobulin. After 48 hours, samples were fixed and stained with nerve-specific antibodies and neuronal outgrowth was quantified. Groups of *thy1-YFP* neurofluorescent mice were anesthetized and received 5 μ L intrastromal injections of either 25 mg/mL bevacizumab or 25 mg/mL nonspecific mouse immunoglobulin. Twenty-four hours after treatment a 2-mm circular corneal epithelial defect was made in each anesthetized mouse, which removed their corneal epithelium and subbasal nerve plexus. Mice were sacrificed 72 hours after wounding, and their corneas were whole mounted. Nerve regeneration in the area of the wound was quantified by fluorescence microscopy.

After 48 hours, growth cultures of dissociated trigeminal neurons showed extensive axon outgrowth, which extended over the surface of each well. Those treated with anti-VEGF antibody showed a 17% reduction in nerve process density compared to control. In living mice, three days after superficial keratectomy, extensive nerve regeneration had already occurred. Mice treated with bevacizumab displayed a 23% reduction in the neuronal density of their subbasal plexus in the area of the wound compared to those treated with nonspecific immunoglobulin. This study suggests that VEGF signaling influences the repair of corneal nerves after injury by demonstrating that abrogation of VEGF signaling reduces nerve growth in vivo and in vitro.

2:30 P.M.

Images of chimerism: Schwann cell migration into peripheral nerve allografts**ELIZABETH L. WHITLOCK**, Washington University School of Medicine

Mentor: Susan E. Mackinnon, M.D., Washington University School of Medicine

■ Severe traumatic peripheral nerve injuries are occasionally reconstructed with a nerve allograft. Systemic immunosuppression is required to allow donor Schwann cells (SCs) to support ingrowth of host axons. Unlike other forms of allografting (e.g., solid organ transplant), however, the immunosuppressive period administered for a peripheral nerve allograft is finite—approximately 18 months in most patients—implying that the donor graft structure is fully repopulated with host SCs by that time. Very little is known about the mechanics of SC repopulation, and understanding the development of chimerism may allow more temporally targeted immunosuppressive regimens.

In these experiments, two transgenic mouse lines with green fluorescent protein conjugated to different SC promoters were used to permit direct visualization of SC migration. These fluorescent transgenic mice were implanted with 5-mm sciatic nerve allografts derived from nonfluorescent, dysgenic Balb/c mice. Mice were immunosuppressed with daily subcutaneous injections of tacrolimus (FK-506, 2 mg/kg/day) or a three-injection course of T cell costimulatory blockade consisting of anti-CD40L and anti-ICOS antibodies and CTLA4 immunoglobulin. To assess SC migration patterns, we conducted serial live imaging of fluorescent SCs every 3 weeks for some mice; all mice were imaged at their harvest endpoint of 6, 10, or 15 weeks. Ten days before sacrifice, the FK-506 regimen was discontinued in some mice to allow rejection of remaining donor SCs, thus allowing us to investigate the effects of both continuous and prematurely discontinued immunosuppressive therapy.

Immune response to different immunosuppressive periods was quantitated with ELISpot for γ -interferon. Explanted grafts were cut into longitudinal or cross-sections and evaluated with immunohistochemistry. Markers evaluated included laminin (SC basal lamina tubes), neurofilament (axon), bromodeoxyuridine (DNA replication), p75 (a SC neurotrophin receptor), and EGR2 (myelination). Anti-S100 was used to differentiate graft (donor) from host SCs. Confocal microscopy was used to colocalize immunohistochemical markers, providing a visual map of the development of chimerism in nerve allografts.

2:45 P.M.

Cerebrospinal fluid-mediated behavior of glioblastoma multiforme: evaluation of invasion, proliferation, and stem cell characteristics**FRANK JOSEPH ATTENELLO**, Johns Hopkins University School of Medicine

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

■ **Introduction:** A subset of malignant glioma cells with increased proliferative capacity, termed brain tumor stem cells (BTSCs), offer a potential cell of origin for brain tumors, including glioblastoma multiforme (GBM). Studies suggest that cerebrospinal fluid (CSF) affects BTSC behavior through potentially direct contact. CSF from cancer patients also displays a unique growth factor makeup. We investigated potential contributions of CSF to GBM-BTSC behavior to further elucidate the tumor-CSF interaction as a potential treatment target.

Methods: From intraoperative tissue, we assayed invasive and proliferative behavior of GBM astrocytes in the presence of CSF collected from patients with and without cancer at 1:10 dilution. Noncancer cortex-derived astrocytes were used as control. Invasion was assayed via the Boyden chamber assay, and proliferation via standard (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The growth of stem cell clusters (neurospheres) was followed by light microscopy for change in size over a one-month period, to evaluate the effect of CSF on tumor stem cells.

Results: Tumor astrocytes exposed to GBM-derived CSF showed a significant increase in both proliferation (MTT signal OD, mean \pm SEM 0.40 \pm 0.02 vs. 0.32 \pm 0.03, $p=0.003$, at 7 days) and invasion (762.5 \pm 17.5 vs. 590.3 \pm 12.3 cells invaded, $p=0.005$) in two separate patient cell lines. Neurospheres formed in growth-optimized media showed a significant decrease in size when subjected to tumor and nontumor CSF over 3 weeks ($p<0.001$, repeated measures ANOVA). This effect was reversed upon return to optimized media in samples incubated in tumor CSF, but size remained constant in samples incubated in nontumor CSF ($p<0.01$, repeated measures ANOVA).

Conclusions: In vitro results suggest GBM cells respond to GBM-derived CSF by increasing their proliferative and invasive capacity. In addition, tumor-derived CSF exerts a nonpermanent inhibitory effect on neurosphere growth versus nontumor-derived CSF. This may provide new therapeutic targets, putatively directed toward BTSCs, for treating a devastating brain cancer.



E. L. WHITLOCK



F. J. ATTENELLO

MONDAY
ROOM B

3:00 P.M.

Registration of a NIRS functional time-series dataset in MRI space

PAUL CAMPION, New York University School of Medicine

Preceptor: Eric M. Wassermann, M.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health



P. CAMPION

■ Near infra-red spectroscopy (NIRS) is a noninvasive method for detecting regional brain activation by measuring the absorption and scattering properties of the oxygenation states of hemoglobin, whose changes are markers of regional cerebral blood flow. Functional magnetic resonance imaging (fMRI), which measures a similar marker, remains the standard tool for functional brain imaging, but NIRS holds an advantage in having higher temporal resolution, much lower complexity, and lower cost. Research in functional imaging with NIRS has been hampered by the difficulty of determining the precise anatomical locations of activations, since many NIRS devices provide only planar data. This

study attempted to cross-validate functional data gathered with NIRS and fMRI in the same subjects. We used a unique device (NIRx Medical Technologies, Brooklyn) that gathers and reconstructs NIRS functional data in three dimensions. We registered the reconstructed NIRS images in MRI space. Then, we compared the registered NIRS functional data to fMRI data for three subjects performing an identical finger-tapping task during data acquisition.

The standard deviation of the distances between the activations measured by both modalities was between 2 and 7 mm. All of the NIRS and fMRI activations were located in the anterior bank of the central sulcus at the “hand knob,” which was expected for the finger-tapping task.

Functional images produced by NIRS can be registered in MRI space with stereotactic guidance. Using this technique, we found close anatomical agreement between the NIRS and fMRI activations produced by finger tapping. Ultimately, we found that NIRS can provide 3-D images that are functionally and anatomically valid, as determined by comparison with fMRI-BOLD.

9:00 A.M.

Cisplatin-based chemotherapy enhances the therapeutic potential of antigen-specific immunotherapy

JORGE A. CABALLERO, Stanford University School of Medicine

Preceptor: James W. Hodge, Ph.D., National Cancer Institute, National Institutes of Health

■ **Purpose:** Lung cancer is the leading cause of cancer death in the United States. Non-small cell lung cancer (NSCLC) accounts for over 85% of new lung cancer cases, half of which present with locally advanced or metastatic disease. Many NSCLC patients will be treated with surgery and an adjuvant platin-based chemotherapy regimen. In spite of therapy, the median survival of patients with metastatic disease is less than 10 months, a fact that underscores the need for novel approaches to treatment for this disease.

Experimental design: It has been shown that certain chemotherapy agents can augment cytotoxic T-lymphocyte (CTL)-mediated antitumor responses. To our knowledge, this effect has not been demonstrated for the drug combinations that are used as standard-of-care for NSCLC or for combination therapies involving recombinant cancer vaccines. Our study explores the potential for biological synergism between a standard-of-care chemotherapy regimen—cisplatin plus vinorelbine—and a recombinant cancer vaccine in the treatment of an orthotopic mouse model of lung cancer.

Results: These studies demonstrate that the standard-of-care chemotherapy combination of cisplatin plus vinorelbine 1) modulates the cell surface expression of immunologically relevant molecules, as well as tumor-associated antigens (TAAs), thereby improving antigen-specific CTL-mediated cytotoxicity in vitro, and 2) enhances antigen-specific T-cell responses in vivo. We also show that the combination of standard-of-care chemotherapy plus immunization with a recombinant yeast-TAA vaccine is superior to either modality alone at reducing tumor burden in a mouse model of lung cancer. Lastly, we examined the translational potential of our preclinical findings by evaluating the effect of this chemotherapy on human NSCLC tumor phenotype and enhancement of antigen-specific CTL-mediated cytolysis in vitro.

Conclusions: These findings suggest potential clinical benefit for the combined use of recombinant yeast vaccine and cisplatin-based chemotherapy regimens.

9:15 A.M.

The protective role of TRAP1 and Hsp90: stress pathways in cancer

ADAM KERN, The University of Chicago Pritzker School of Medicine

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

■ Heat shock protein 90 (Hsp90) is a molecular chaperone that protects cells from oxidative stress. The family of Hsp chaperones includes cytosolic and mitochondrial Hsp90 and the mitochondrially localized paralog TRAP1. Heat shock proteins stabilize over 100 “client” proteins, many of which are involved in stress response pathways induced in cancer. Hsp90 can be pharmacologically blocked, leading to decreased chaperone cycling and proteasome-mediated client protein degradation. TRAP1 has been shown to be phosphorylated and activated by PINK1. Impairment of PINK1 is implicated in the pathogenesis of mitochondrial-mediated central nervous system disease. TRAP1 and Hsp90 are implicated in protecting cells from mitochondrial-mediated apoptosis induced by oxidative stress in cancers and nonpathologic brain and testicular tissues. Interestingly, solid tumors, brain, and testes all rely on aerobic glycolysis and are prone to oxidative stress. However, mitochondrially targeted Hsp90 inhibitors are only reported to induce apoptosis in tumors and not in the brain and testes. We hypothesize that TRAP1 and Hsp90 act in common in certain tissues to chaperone mitochondrial protein and conditionally suppress cyclophilin-mediated apoptosis. The differential susceptibility of these tissues to reactive oxygen species (ROS) and mitochondrially targeted Hsp90 inhibitors may be a function of varying expression of mitochondrial Hsp90 and phosphorylated TRAP1.

We profiled the expression of PINK1, TRAP1, and associated proteins in a panel of normal and pathologic tissues and cell lines. We identified certain lines with increased sensitivity to targeted Hsp90 inhibition and pharmacologically induced ROS and assayed TRAP1 phosphorylation status in vitro. We then cotransfected PINK1 and TRAP1 fusion proteins in a HeLa cell model, assayed TRAP1 phosphorylation status under control and induced ROS treatment conditions, and correlated drug sensitivity with cell viability as a function of TRAP1 phosphorylation. Our data are consistent with a protective role for TRAP1 and Hsp90 against mitochondrial ROS, and suggest both an explanation of the pathophysiology of, and therapeutic modalities for, certain cancers.



J.A. CABALLERO



A. KERN

MONDAY
AUDITORIUM

9:30 A.M.

Glycogen synthase kinase 3 (GSK-3) as a potential target for acute myeloid leukemia (AML) differentiation**LORETTA S. LI**, Harvard Medical School

Mentor: Kimberly Stegmaier, M.D., Dana-Farber Cancer Institute and Harvard Medical School

■ Despite high-dose chemotherapy, the majority of patients diagnosed with acute myeloid leukemia (AML) ultimately succumb to the disease. New treatment approaches are needed. Kinases have emerged as potential therapeutic targets for AML, with inhibition leading to cell death and/or differentiation. The feasibility of differentiation therapy for AML was demonstrated with the use of all-trans retinoic acid for acute promyelocytic leukemia. However, identification of the myeloid differentiation state is not easily amenable to traditional phenotypic screening. We applied a novel approach to high-throughput screening in which gene expression signatures define the AML versus mature myeloid states. We used this signature-based approach in two parallel screens to evaluate kinase involvement in AML differentiation: small-molecule library and high-throughput RNA interference (RNAi) screening.

A 32-gene signature distinguishing AML from mature myeloid cells was developed. We screened 84 kinase inhibitors, in two AML cell lines, for new agents that induce the myeloid differentiation signature. Four of the top hits from the screen have activity against glycogen synthase kinase 3 (GSK-3). Similarly, GSK-3 scored as the top hit in the RNAi screen, which sought to identify those kinases whose genetic loss induces the AML differentiation signature. These findings suggest a new role for GSK-3 as a potential therapeutic target in AML, perhaps surprising results given GSK-3's reported inhibition of sonic hedgehog and Wnt signaling. Further validation of our results is therefore critical.

We are currently validating the GSK-3 inhibitor and shRNA hits using the original 32-gene differentiation signature. Next, we will determine the role of GSK-3 inhibition in more traditional differentiation assays and its effects on cell growth. Future studies will address the role of GSK-3 inhibition on its downstream signaling pathways in AML. Our ultimate goal is the translation of compelling preclinical findings to clinical testing.



L. S. LI



M. P. CHAO

9:45 A.M.

Targeting CD47 eliminates human acute myeloid leukemia stem cells by disrupting a mechanism of immune evasion**MARK P. CHAO**, Stanford University School of Medicine

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

■ The long-term prognosis of patients with acute myelogenous leukemia (AML) is extremely poor. A permanent cure requires elimination of the leukemic stem cell (LSC), the only cell population capable of initiating and maintaining leukemic disease. Therefore, the identification of unique LSC markers is essential for their targeted destruction. We have identified CD47 as a cell surface protein with high expression on human AML LSCs. Interestingly, the main function of CD47 is inhibition of immune phagocytosis by macrophages through binding its macrophage ligand Sirp α . In mouse leukemia models, upregulation of CD47 is necessary for leukemogenesis. Thus, leukemic pathogenesis appears to be partly regulated by evading immune phagocytosis through upregulation of CD47. Using a monoclonal antibody, we investigated whether disrupting the CD47-Sirp α interaction could eliminate LSCs by macrophage phagocytosis.

We generated a monoclonal antibody against human CD47 that specifically blocks the CD47-Sirp α interaction. This anti-CD47 antibody was administered in vitro to leukemia cell lines and LSCs isolated from four human AML patients in a macrophage-containing cell culture. High CD47-expressing cell lines as well as LSCs from all four AML patients underwent significant phagocytosis with treatment of anti-CD47 antibody compared to isotype controls. In vivo, coating LSCs with anti-CD47 antibody prior to xenotransplantation into NOG immune-deficient mice blocked disease engraftment while isotype controls did not. Moreover, preliminary results demonstrate that in vivo administration of anti-CD47 antibody to AML-engrafted NOG mice significantly decreases the presence of their leukemic disease.

These data indicate that disrupting CD47 function in human AML can eliminate leukemic disease both in vitro and in vivo. Given the ability to eliminate the cancer stem cell population, an antibody-based therapy blocking CD47 function could have a profound effect as a potential cure for leukemia.

10:00 A.M.

In vivo and in vitro characterization of the role of NF2 in wound healing**SANDY MONG**, Harvard Medical School

Mentors: Andrea McClatchey, Ph.D., and Dennis Orgill, M.D. Ph.D., Brigham and Women's Hospital and Harvard Medical School

■ The *NF2* tumor suppressor gene encodes for the protein Merlin, a band 4.1 superfamily member that is closely related to the membrane-organizing proteins ezrin, radixin, and moesin. Merlin is unique among tumor suppressors for being localized at the interface between the cortical actin cytoskeleton and the extracellular milieu. Merlin is involved in controlling proliferation and migration during development in multiple epithelial tissues by regulating transmembrane receptors that are linked to both wound healing and cancer. Given these observations, we hypothesized that Merlin would play a role in wound healing.

We utilized a *Nf2* conditional knockout model in which a tamoxifen-inducible keratin 14 promoter drives expression of Cre in the epidermis of adult mice. Wound closure was accelerated in the tamoxifen-treated *Nf2^{lox/lox};K14cre^{er}* animals as compared to oil-treated control animals. Up to threefold greater proliferation was present in the leading edges of tamoxifen-treated versus control wounds, with the latter demonstrating both basal and suprabasal keratinocyte proliferation extending farther from the wound bed into unwounded skin, as compared to predominantly basal cell proliferation in wounded control animals.

To determine whether proliferation or migration underlies the phenotype, we conducted in vitro scratch assays. Keratinocytes were triggered to slow proliferation and initiate differentiation with calcium prior to scratch initiation. Rates of gap closure were accelerated in Merlin knockdown and dominant-negative keratinocytes versus cells with endogenous and exogenous Merlin. This demonstrates that *Nf2* depletion in vitro accelerates migration of keratinocytes independent of the proliferative advantage observed in vivo.

This study demonstrates that *Nf2* depletion may both potentiate cell motility in vitro as well as confer an early proliferation advantage to enhance wound healing in vivo. These studies provide groundwork for further studies delineating the independent functions of Merlin in both cell migration and proliferation.

10:15 A.M.

Regulation of epithelial genomic instability by stromal fibroblasts in breast carcinogenesis**CYNTHIA ANN JIMENEZ**, University of California, San Francisco, School of Medicine

Mentor: Thea Tlsty, Ph.D., University of California, San Francisco

■ Epithelial breast cancers develop in a microenvironment regulated by extracellular matrix proteins and stromal cells. Identifying fibroblast-epithelial interactions is essential to understanding a complete picture of breast tumor progression. Genomic instability is an important aspect of carcinogenesis that may be regulated by fibroblast-epithelial interactions. We sought to determine if fibroblasts can regulate epithelial genomic instability in a 3D coculture system. This novel system allows fibroblasts and epithelial cells to be cultured within adjacent, yet distinct, layers of matrigel. To understand both positive and negative interactions on genomic instability, we performed cocultures of premalignant epithelial cells with carcinoma-associated fibroblasts (CAF) and cocultures of malignant epithelial cell lines with normal fibroblasts. Genomic instability was determined by assaying for gene amplification and aneuploidy. Gene amplification was measured with the PALA assay, and aneuploidy was measured with propidium iodide staining for DNA content. In summary, we utilized a novel 3D coculture system to analyze the effects of fibroblasts on epithelial genomic instability in breast carcinogenesis.



S. MONG



C. A. JIMENEZ

CANCER BIOLOGY I

MONDAY
AUDITORIUM

10:30 A.M.

The study of pulmonary metastases using a novel ex vivo lung organ culture assay

M. ALI KHAN, University of California, Los Angeles, David Geffen School of Medicine at UCLA

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

■ Pulmonary metastasis remains a significant problem for many cancer patients. To improve our understanding of metastasis biology and evaluate new therapies, we sought to develop an ex vivo lung culture assay amenable for the study of metastasis in a microenvironment that mimics in vivo conditions. Such an assay would serve as a needed intermediary between currently available in vitro and in vivo metastasis assays, and allow the study of metastasis as both an active process as well as a physical entity. Distinct mice were injected intravenously with green fluorescent protein (GFP)-labeled tumor cells. The mice were then euthanized, and the lungs extracted, fixed in agar, and sectioned for culture in supplemented serum-free conditions resting on sterile gel-foam at an air-culture interface. Sections were imaged at day 0, 3, 7, 10, and 14 using an inverted fluorescent video microscope, and software was used to determine the number and size of cellular events.

Routine histology of cultured sections showed maintenance of the lung architecture, though at reduced cellularity. Fluorescent imaging at day 0 revealed single tumor cells within the lung parenchyma, a select minority of which proliferated into distinct metastatic clusters visible on imaging at day 14. Unlike conventional in vitro assays, the lung culture assay successfully predicted the metastatic phenotype observed in vivo in six of six tested high-versus-low metastatic cell line variant pairs. Efforts to describe the mechanism of cell death and proliferation within the assay are currently underway, as are experiments utilizing the assay as a drug screening tool.

These studies support the lung culture assay as a tool for the study of metastatic phenotype, and potentially to evaluate therapeutic strategies directed against metastases and metastatic progression.



M. A. KHAN



S. WEINER

CANCER BIOLOGY II

1:30 P.M.

Xenotropic murine leukemia virus-related virus: a possible role of envelope proteins in chronic inflammation of the prostate

SHOSHANA WEINER, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentors: Robert H. Silverman, Ph.D., and Eric A. Klein, M.D., Glickman Urological and Kidney Institute, Cleveland Clinic

■ RNase L, an endoribonuclease involved in viral defense, has been inconclusively linked to prostate cancer. A new gammaretrovirus, xenotropic murine leukemia virus-related virus (XMRV), was detected in prostate tumors of men who carry two copies of the R462Q variant of RNase L more frequently than in heterozygous and wild-type men. We hypothesize that XMRV may facilitate prostate cancer progression by causing local inflammation, and that the envelope proteins of XMRV may be important mediators of this response. To examine this hypothesis, we planned to characterize the effects of XMRV envelope protein expression on the histology and pro-inflammatory response of mouse prostate tissue using a lentiviral expression system. We further planned to characterize the pro-inflammatory response of the XMRV envelope protein using ELISAs to detect the production of pro-inflammatory cytokines by peripheral blood mononuclear cells treated with envelope proteins.

Currently, the gp70 subunit of XMRV envelope has been produced in mammalian cell culture using a lentiviral expression system, but further work is needed to optimize purification. Preliminary cell culture work in mouse fibroblast cells has resulted in the expression of control GFP protein, but not XMRV envelope protein, as detected by Western blot.

In conclusion, more work needs to be done to refine envelope protein production and purification, and a different system may need to be employed in order to express envelope proteins in vivo.

1:45 P.M.

Prognosis in esophageal cancer patients by expression of six cytokine genes

GIANG HUONG NGUYEN, Albany Medical College

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

■ **Purpose:** Adenocarcinoma of the esophagus demonstrates a different histology from squamous carcinoma, and the prognostic factors for this histology type are poorly defined. Recent studies have indicated that a unique cytokine gene signature is shown to predict hepatocellular carcinoma and lung adenocarcinoma prognosis. We investigated whether the expression levels of these cytokines within tumors and its matched nontumor tissues from esophageal cancer patients could influence patient outcome.

Patients and methods: Two hundred and eighteen resected carcinoma and noncarcinoma tissues of the esophagus, 132 adenocarcinomas, and 86 squamous carcinomas were analyzed for a panel of 23 cytokines using real-time quantitative reverse transcription polymerase chain reaction. Analyses of cytokine expressions with survival outcome were performed using Cox proportional hazards models, Kaplan-Meier methods, and log-rank test.

Results: The 23-gene signature was grouped into two cohorts based on prognostic values independent of lymph node status (adenocarcinomas, $p=0.024$) using unsupervised hierarchical clustering. High tumor over nontumor ratio (TNR) expression of four pro-inflammatory cytokines ($\text{TNF}\alpha$, $p=0.03$; $\text{IFN}\gamma$, $p=0.05$; $\text{IL-1}\alpha$, $p=0.01$; and $\text{IL-1}\beta$, $p=0.03$) and two others (PRG, $p=0.02$; HLA-DPA, $p=0.05$) was shown to correlate with poor prognosis in the adenocarcinoma patients in both the univariate and multivariate analysis. In addition, expression of (PRG, $p=0.01$; $\text{IL-1}\alpha$, $p=0.03$; $\text{IL-1}\beta$, $p=0.02$) was individually associated with a survival disadvantage on univariate analysis on the Barrett's associated adenocarcinomas. In contrast, elevated expression of IL-18 ($p<0.01$) corresponded with an unfavorable prognosis in the sporadic adenocarcinomas. There was no association of these cytokines found with the squamous carcinomas.

Conclusions: We have identified a unique cytokine gene signature that is an independent marker of outcome in patients with resected adenocarcinoma of the esophagus by RT-PCR, a technology that can be easy to implement in clinical laboratories. Our study may also contribute to advance the knowledge of the molecular relationship between cytokines and esophageal carcinogenesis.

2:00 P.M.

The efficacy of selective nuclear factor- κ B inhibitors in cancer cachexia

ASHLEY WYSONG, Duke University School of Medicine

Mentors: Albert Baldwin, Ph.D., and Marion E. Couch, M.D., Ph.D., University of North Carolina at Chapel Hill School of Medicine

■ Cancer cachexia is a severe wasting syndrome characterized by the progressive loss of lean body mass and systemic inflammation. Cachexia is seen in as many as 80% of patients with advanced malignancy and accounts for up to 30% of cancer-related deaths. Activation of nuclear factor- κ B (NF- κ B) by pro-inflammatory cytokines elevated in cancer cachexia has been shown to induce muscle atrophy. In this study, we investigated two selective NF- κ B inhibitors, NEMO binding domain (NBD) peptide and Compound A, which target subunits of the upstream I κ B kinase (IKK) complex. Experiments were carried out in vivo in a murine model of colon (C-26) adenocarcinoma and in vitro in C-26 adenocarcinoma cells and C2C12 myocytes. Approximately one hundred BALB/c mice were inoculated with C-26 cells to induce tumor burden and cachexia.

Mice that received selective NF- κ B inhibitors lost significantly less weight than untreated tumor-bearing mice, and had improved lean muscle mass and fat mass by magnetic resonance imaging (MRI). A reduction in skeletal muscle atrophy was confirmed with increased gene expression of MuRF-1 and MAFbx/atrogin-1. In addition, tumor-bearing mice treated with Compound A or NBD peptide had significantly greater heart masses and improved heart function as shown by echocardiography when compared to untreated tumor-bearing mice.

Selective NF- κ B inhibition shows therapeutic promise for cancer-induced wasting of skeletal and cardiac muscle. Further studies must be undertaken to confirm these findings and to further elucidate the mechanisms involved in cancer cachexia.



G. H. NGUYEN



A. WYSONG

MONDAY
AUDITORIUM

2:15 P.M.

Attenuated transforming growth factor- β signaling promotes nuclear factor- κ B activation in head and neck cancer

JONAH COHEN, The Warren Alpert Medical School of Brown University

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

■ Aberrant activation of nuclear factor- κ B (NF- κ B) has been implicated in promoting cell proliferation, survival, angiogenesis, and malignant progression of human cancers, including head and neck squamous cell carcinoma (HNSCC). Transforming growth factor- β (TGF- β) is a potent inhibitor of proliferation, and epithelia deficient in TGF- β receptor II (TGF- β RII) are strongly susceptible to malignant transformation. Recently, we identified reduced expression of TGF- β RII in a panel of ten HNSCC (UM-SCC) cell lines by genome-wide microarray analysis. Deficient TGF- β RII expression in the panel of UM-SCC lines was confirmed by real-time RT-PCR and Western blot analysis. We observed attenuated TGF- β signaling, evidenced by partial or complete loss of response to TGF- β -mediated growth arrest, as well as decreased expression of TGF- β target genes when compared to normal controls. Additionally, deficient TGF- β RII expression was associated with accumulation of mutant p53 in a subset of UM-SCC lines, and siRNA knockdown of p53 enhanced TGF- β RII gene expression. In UM-SCC lines, excess recombinant TGF β or forced expression of TGF- β RII enhanced TGF- β reporter gene activity. Restoration of TGF- β signaling inhibited both basal and TNF- α -induced NF- κ B activation, indicating that reduced expression of TGF- β RII contributes to enhanced constitutive and inducible NF- κ B transcriptional activity in a subset of HNSCC expressing mutant p53. Reduced expression of TGF- β RII and increased phosphorylated nuclear NF- κ B was also observed in human HNSCC tissue specimens when compared with normal mucosa by immunohistochemistry (IHC). Further, in a murine model of HNSCC induced by conditional TGF- β RII deletion in aerodigestive epithelia, carcinomas exhibited a significant increase in NF- κ B nuclear translocation by IHC and increased expression of NF- κ B target genes. Together, these findings suggest a novel molecular framework whereby TGF- β RII deficiency in HNSCC with mutant p53 may promote cancer growth and progression by augmenting NF- κ B activation in the setting of chronic inflammation and TNF induction.



J. COHEN



R. EHSANIAN

2:30 P.M.

The role of Yes-associated protein in head and neck cancer

REZA EHSANIAN, Stanford University School of Medicine

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

■ YAP (Yes-associated protein), a 65-kDa protein, has been recognized as a component of a highly conserved pathway regulating apoptosis and proliferation in *Drosophila* and has been demonstrated to have potent oncogenic effects in hepatocellular carcinoma. This seems in direct contradiction of studies in which YAP acts as a transcription cofactor for the p53 family member p73, mediating the expression of cell death-promoting genes and inducing apoptosis. In studying gene signatures of head and neck cancers, we observed YAP overexpression by microarray expression profiling in murine and human SCC lines. We analyzed YAP gene and protein expression in HNSCC lines and tumor specimens. We observed that it is overexpressed in the subset of SCC lines with decreased p63 and p73 levels. Conversely, expression of YAP is decreased in HNSCC overexpressing p63 and p73. Knockdown c-Rel, known to interact with p63, and p63 itself had the strongest effect in promoting YAP expression. Interestingly, p73 knockdown also promoted YAP expression. These findings provide evidence that YAP overexpression is due to aberrant transcriptional activation of p63, c-Rel, and p73 in cells deficient for p53, while such expression is repressed in cells overexpressing mt p53, with decreased levels of p63 and p73. Our data suggest that the expression of YAP is strongly regulated by p63, c-Rel, and p73. Interestingly, in cell lines with high YAP levels much of the YAP is confined to the cytoplasm. Increasing the amount of nuclear YAP increased the amount of apoptosis in these cell lines. In cell lines where YAP levels were greatest in the nucleus, p63 levels were high and hence overall YAP levels were low. We show that increasing nuclear YAP along with knockdown of p63 increases the rate of apoptosis in our cell lines. We propose that YAP may not be able to serve as a pro-apoptotic cofactor in cells where YAP is restricted to the cytoplasm, or when its expression is repressed and activity inhibited by p63. Therefore, in the analysis of tissue samples by IHC, the levels and localization of YAP along with p63 and p73 protein levels must be assessed to determine the inhibited ability of YAP to promote apoptosis.

2:45 P.M.

Human papillomavirus type 16 E6-mediated PTPN13 loss synergizes with MAPK signaling downstream of ErbB2 to allow invasive growth in head and neck squamous cell carcinogenesis

ANDREW C. HOOVER, University of Iowa Roy J. and Lucille A. Carver College of Medicine

Mentor: John H. Lee, M.D., University of Iowa Roy J. and Lucille A. Carver College of Medicine and Veterans Affairs Medical Center

■ Infection with oncogenic subtypes of human papillomavirus (HPV) is a causative factor in 25% of squamous cell head and neck cancers (HNSCCs) and 60% of tonsillar SCCs. Malignant transformation is associated with overexpression of the viral oncoprotein, E6. E6 from oncogenic HPV subtypes contains a c-terminal PDZ binding motif, which is absent in non-oncogenic forms. Although required for an invasive phenotype, the mechanism through which this motif promotes carcinogenesis was unknown. We recently showed that the E6 PDZ motif physically associates with and degrades the

protein tyrosine phosphatase PTPN13. And, in a C57BL/6 mouse model of tonsillar SCC, E6- or shRNA-mediated PTPN13 loss synergizes with overexpression of H-RAS^{V12} during invasive growth in vivo.

Although RAS is an uncommon HNSCC oncogene, ErbB2 is overexpressed in up to 47% of these cancers and is an upstream activator of the RAS/RAF/MAPK pathway. Therefore, we examined whether PTPN13 loss synergizes with ErbB2 for invasive growth. We show that 1) like H-RAS^{V12}, ErbB2 overexpression synergizes with PTPN13 loss to allow invasive growth of mouse tonsil cells, 2) PTPN13 loss correlates with enhanced MAPK signaling in epithelial cell lines, 3) PTPN13 regulates MAPK signaling by acting between RAS and MEK, 4) the phosphatase domain of PTPN13 is required for this regulation, and 5) the MEK inhibitor U0126 abrogates MAPK signaling in epithelial cells that overexpress ErbB2 and have lost PTPN13. These findings determine how PTPN13 loss synergizes with cellular oncogenes during invasive growth and suggest a potential therapeutic intervention based on an E6 mechanism.



A. C. HOOVER

MONDAY
ATRIUM

POSTER A1

Development and characterization of CD34-conjugated nanoparticles and a magnetic bone marrow biopsy for evaluating acute leukemia

JASON E. JAETAO, University of New Mexico School of Medicine

Mentor: Richard S. Larson, M.D., Ph.D., University of New Mexico School of Medicine

■ Acute leukemia is a malignant cancer of the blood and bone marrow and a disease in which early detection is key to improving survival. The current standard for diagnosis and reevaluation of leukemia is a bone marrow biopsy. The absolute and relative detection sensitivities of a standard bone marrow biopsy are approximately 1×10^5 blast cells and 1% blast cells, respectively. This limitation is due to nontargeted sampling. The use of antibody-conjugated superparamagnetic iron oxide (SPIO) nanoparticles against acute leukemia antigens coupled with a “magnetic needle” presents a powerful option for targeted sampling. Both high- and minimal-CD34-expressing cell lines were used in this study with anti-CD34-conjugated SPIO nanoparticles. Various time-course conditions were used to ascertain optimal binding parameters. Microscopy, superconducting quantum interference device (SQUID) magnetometry, and in vitro magnetic needle extraction were used to assess nanoparticle-cell binding. The CD34-conjugated nanoparticles were shown to preferentially bind high-CD34-expressing cell lines as identified with Prussian blue-aided microscopy and SQUID measurements. The newly developed magnetic needle exhibited the capacity to isolate CD34-positive cell lines from nonmalignant cells from peripheral blood in vitro. Furthermore, the magnetic needle collected blast cells bearing magnetic nanoparticles at relative levels of below 5×10^4 cells and absolute levels of below 0.7%, which, at the minimum, meet levels of conventional bone marrow biopsy. These data suggest the potential to increase the sensitivity of bone marrow biopsy using antigen-targeted magnetic nanoparticles and a magnetic needle for the evaluation and diagnosis of acute leukemia.



J. E. JAETAO



A. MALHOTRA

POSTER A2

Developmental mimicry in the metastatic niche: modeling leukemic metastasis in vitro and in vivo

AJAY MALHOTRA, Keck School of Medicine of the University of Southern California

Mentor: Shahin Rafii, M.D., Howard Hughes Medical Institute, Weill Cornell Medical College

■ Establishment of functional vasculature is a prerequisite for tumor growth; this fact has been exploited by therapeutic strategies targeting neovessels within advancing tumors. Leukemia, a cancer of blood-derived cells, can occasionally form solid tumor masses, and these require the formation of neovasculature. Previous work has shown that melanoma can transdifferentiate to lumenally incorporated cells in neovessels. We hypothesize that leukemic cells have a similar potential to develop into endothelial cells (ECs) and this supports tumor growth by providing an intrinsic source of tumor vessels. These leukemia-derived ECs could also function by interacting with endogenous endothelium, either to exit the vasculature en route to a site of metastasis or to enter a dormant/quiescent niche in the bone marrow. We have identified a subset of the human erythroleukemia cell line (HEL 92.1.7) that shows expression of VE-Cadherin transcript and protein and binds to human umbilical vein endothelial cells (HUVECs) in vitro. We have also shown that these HEL-VECad⁺ cells localize to the branch points of host vessels in murine tumor xenografts. These data support the hypothesis that expression of VE-Cadherin by HEL has a functional role and may mediate the development of solid tumor masses through productive interactions within tumor neovasculature.

POSTER A3

The role of KiSS-1 in type III TGF- β receptor-mediated metastasis suppression

MICHAEL SANGMIN LEE, Duke University School of Medicine

Mentor: Gerard C. Blobe, M.D., Ph.D., Duke University School of Medicine

■ Transforming growth factor-beta (TGF- β) is a cytokine critical in development, proliferation, and differentiation, with alterations in TGF- β receptor expression and signaling frequently involved in cancer development and progression. TGF- β has a dichotomous role in cancer progression, at first suppressing cancer initiation but then enhancing cancer progression. The type III TGF- β receptor (T β RIII) is a TGF- β coreceptor that is highly expressed in normal tissues but is lost during cancer progression. Restoring T β RIII expression in breast cancer cells decreases invasion and metastasis through mechanisms that remain to be defined. KiSS-1 is a putative metastasis suppressor with an unclear role in breast cancer progression. We have identified KiSS-1 as a gene whose expression is selectively repressed by T β RIII in breast cancer. To determine whether KiSS-1 was a potential mediator of the antimetastatic effect of T β RIII, we explored the function of KiSS-1 in breast cancer models. Using quantitative reverse transcription PCR, we established that malignant breast cell lines express significantly higher levels of KiSS-1 mRNA than benign breast epithelial cell lines. In the MDA-MB-231 breast cancer line stably transfected with T β RIII, KiSS-1 expression was significantly decreased by TGF- β_1 in the T β RIII-overexpressing cells relative to mock-transfected controls. Functionally, gain-of-function (through overexpression of KiSS-1) and loss-of-function (through shRNA-mediated knockdown of KiSS-1 expression) studies and their effects on the proliferation, migration, and invasion of MDA-MB-231 breast cancer cells are underway. These studies will define the role of KiSS-1 in breast cancer progression and whether KiSS-1 mediates the tumor suppressor effects of T β RIII.

POSTER A4

Aberrant JAK2 transcription and miRNA dysregulation in spontaneous STAT1^{-/-} murine mammary adenocarcinoma

CHARLES RICKERT, Washington University School of Medicine

Mentor: Robert D. Schreiber, Ph.D., Washington University School of Medicine

■ In experiments studying tumor immunosurveillance, we observed that STAT1^{-/-} mice spontaneously develop mammary adenocarcinomas. This mammary tumor shares many characteristics with human breast cancer and is a potentially useful model. Investigation into the mechanism of tumorigenesis identified that the STAT1^{-/-} mammary tumors exhibit JAK2 hyperactivation and overexpression. Additionally, STAT3 and STAT5 are constitutively active. Constitutive STAT3 phosphorylation is the result of the hyperactive JAK2. Further work with this novel murine mammary adenocarcinoma demonstrated that tumor cell survival requires the constitutive activation of the JAK2/STAT3 pathway.

Here, we systematically investigate the mechanism of JAK2 overexpression in the STAT1^{-/-} tumors by examining the control of JAK2 gene expression at the genomic, transcriptional, and translational levels. We rule out the possibility of gene amplification or increased JAK2 mRNA stability. We unequivocally establish that JAK2 mRNA in STAT1^{-/-} is overexpressed due to increased JAK2 transcription. As measured by real-time PCR, unspliced JAK2 mRNA is dramatically elevated in STAT1^{-/-} tumors, suggesting that JAK2 overexpression results from an increase in JAK2 transcription. Using a nuclear run-on assay, increased JAK2 mRNA transcription is confirmed. To further characterize dysregulation in STAT1^{-/-} tumors, we performed an miRNA expression profile. Approximately 10% (43/499) of miRNA in STAT1^{-/-} tumor cell lines are dysregulated. Additionally, miR-433, which is postulated to play a role in regulation of JAK2, is decreased in primary STAT1^{-/-} tumors and tumor cell lines. Investigation is ongoing to examine the connection between dysregulation of miRNA and the tumor phenotype. Furthermore, a determination of STAT5's role in tumorigenesis is underway.



M. S. LEE



C. RICKERT

POSTER A5

Identifying the CA125 binding domain of mesothelin

OSAMU FERNANDO KANEKO, University of California, Los Angeles, David Geffen School of Medicine at UCLA

Preceptor: Ira Pastan, M.D., National Cancer Institute, National Institutes of Health

■ Ovarian cancers very frequently express high levels of the mucin CA125 (also known as MUC16) and mesothelin, a 40-kD GPI-linked cell surface glycoprotein also found on the normal peritoneal and pleural mesothelium. Because CA125 strongly binds to mesothelin, it has been hypothesized that the interaction between CA125 on shed ovarian cancer cells and mesothelin on the peritoneal surface facilitates the implantation and peritoneal spread of ovarian cancer. CA125-mesothelin binding between homotypic ovarian cancer cells may also lead to the formation of tumor aggregates. To identify the CA125 binding domain of mesothelin, we generated truncated mutants of mesothelin and assessed their binding capability to CA125. Fc-fusion proteins of different regions of mesothelin were produced by transfection of appropriate plasmids into HEK 293T cells and purification of the Fc-mesothelin fragments over a protein A column. Binding was assessed with flow cytometry, sandwich ELISA, and Western blotting. Although the mature form of mesothelin contains 295 amino acids, a fragment consisting of 64 amino acids showed nearly full binding capacity to CA125 when compared with full-length mesothelin. Smaller fragments from within this region showed no binding to CA125. We are currently carrying out alanine scanning mutagenesis to identify critical amino acids involved in the interaction. Characterizing the interaction between mesothelin and CA125 will allow a better understanding of the progression of ovarian cancer and may also lead to therapeutics that can prevent or reverse its peritoneal spread.



O. F. KANEKO



C. P. GIACOMINI

POSTER A6

Identification and characterization of *TMPRSS2/ERG* transcriptional targets in prostate carcinogenesis

CRAIG P. GIACOMINI, Stanford University School of Medicine

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

■ Prostate cancer is a remarkably common and often devastating disease. Recent studies indicate that the majority of prostate cancers harbor gene fusions between the 5' untranslated region of the androgen-responsive *transmembrane protease, serine 2 (TMPRSS2)* gene and a member of the ETS family of transcription factors, most commonly *v-ets erythroblastosis virus E26 oncogene homolog (avian) (ERG)*. However, the functional contribution of *TMPRSS2/ETS* to prostate cancer development and progression is not yet well understood. The goal of this study was to characterize the role of *TMPRSS2/ERG* in prostate tumorigenesis. Toward this goal, we analyzed transcriptional and genomic profiles of 64 prostate tumors to identify *TMPRSS2/ETS*-associated expression signatures and cooperating genetic alterations. We are also creating a tetracycline-inducible system for the controlled expression of *TMPRSS2/ERG* in prostate epithelial cells.

Our transcriptional profiling, genomic profiling, and RT-PCR analysis of prostate tumors confirmed the presence of *TMPRSS2/ERG* in the majority of prostate cancers studied. *TMPRSS2/ERG* was associated with a previously identified gene-expression subtype ("subtype-2"), characterized by an "invasion/angiogenesis" gene-expression pattern, frequent deletion of 8p21 (*NKX3-1*), and an increased rate of tumor recurrence. Consistent with this finding, subtype-2 expression patterns were also enriched for genes with putative ETS binding sites in or near their promoter sequences. Taken together, these findings support a relationship between *TMPRSS2/ERG* and tumor aggressiveness in prostate cancer. In addition, we have cloned *TMPRSS2/ERG* from a clinical specimen and are near to completing a system for the regulatable expression of *TMPRSS2/ERG* in immortalized but nontumorigenic RWPE-1 prostate epithelial cells. This system will permit the identification of *TMPRSS2/ERG* direct transcriptional targets and study of the functional connection between *TMPRSS2/ERG* and tumor aggressiveness. Elucidating the role of *TMPRSS2/ERG* in prostate carcinogenesis may provide new opportunities to treat this devastating disease.

POSTER A7

Combining targeted antivascular gene therapy with multiple compounds directed against the tumor microenvironment inhibits tumor growth

JOHN S. QUICK, New York University School of Medicine

Preceptor: Steven K. Libutti, M.D., National Cancer Institute, National Institutes of Health

■ The establishment of a vascular supply is crucial for the survival and growth of solid tumors. Compared to normal vasculature, endothelial cells lining tumor blood vessels are characterized by an overexpression of α_v integrins on their luminal surface. This tumor-specific vascular “address” can be exploited by vectors delivering a variety of cytotoxic agents. A novel hybrid vector, adeno-associated virus/phage (AAVP), is composed of a single-stranded filamentous bacteriophage and the eukaryotic gene expression cassette of adeno-associated virus. The bacteriophages, which naturally lack tropism for mammalian cells, are engineered to target α_v integrins by the addition of an arginine-glycine-aspartic acid (RGD) peptide to their protein capsid. Binding of this RGD homing peptide to α_v integrin leads to integrin-mediated endocytosis of the AAVP and subsequent transgene expression in tumor endothelial cells.

Our lab has constructed an RGD-AAVP expressing the gene for tumor necrosis factor- α (RGD-AAVP-TNF- α). In nude mice bearing a subcutaneous human melanoma xenograft, RGD-AAVP-TNF- α specifically targeted tumor vasculature and elicited a potent antitumor effect with no observable systemic toxicity.

Upon completion of RGD-AAVP-TNF- α treatment, we plan to demonstrate that subsequent administration of agents that target multiple elements of the tumor microenvironment can sustain the antitumor effect in this murine model. Specifically, lenalidomide, an immunomodulatory drug; sunitinib, a tyrosine kinase inhibitor; and metronomic cyclophosphamide, a cytotoxic chemotherapeutic, were chosen in an effort to inhibit compensatory proangiogenic pathways as the tumor seeks to reestablish its vascular supply following RGD-AAVP-TNF- α treatment. Through additional studies in mice bearing human tumor xenografts, we aim to test the safety and efficacy of this sequential two-hit regime in hopes of developing more effective treatment strategies for cancer patients.

POSTER A8

Low-field paramagnetic resonance imaging of glycolytic activity and tumor oxygenation following anti-angiogenic therapy in mice

SONNY BATRA, Stanford University School of Medicine

Preceptor: James Mitchell, Ph.D., National Cancer Institute, National Institutes of Health

■ A priori knowledge of spatial and temporal changes in oxygenation in solid tumors, a key prognostic factor in cancer treatment outcome, could greatly improve treatment planning in radiotherapy and chemotherapy. Pulsed electron paramagnetic resonance imaging (EPRI) provides quantitative three-dimensional maps of tissue oxygenation (pO₂) in living objects. In this study, we implemented an EPRI setup that could acquire pO₂ maps in almost real time for two dimensions and in minutes for three dimensions. We also designed a combined EPRI and MRI system that enabled generation of pO₂ maps with anatomic guidance. Using EPRI and an air/Carbogen (95% O₂ plus 5% CO₂) breathing cycle, we visualized perfusion-limited hypoxia in murine tumors. The relationship between tumor blood perfusion and pO₂ status was examined, and it was found that significant hypoxia existed even in regions that exhibited blood flow. In addition, high levels of lactate were identified even in normoxic tumor regions, suggesting the predominance of aerobic glycolysis in murine tumors. Our technique can be exploited to follow changes in tumor oxygen levels after treatment with modulators of anti-angiogenic therapy. We have been treating murine SCC7 tumors with vascular endothelial growth factor receptor tyrosine kinase inhibitors and using EPRI to follow changes in oxygen tension. We also used dynamic contrast enhanced MRI to measure changes in tumor blood perfusion. We expect to determine whether a window of normalized oxygen and blood perfusion to the tumor environment exists for directing combination radiotherapy and/or chemotherapy. This report presents a rapid noninvasive method to obtain quantitative maps of pO₂ in tumors, reported with anatomy. In addition, this method may also be useful for studying the relationship between oxygenation status and tumor-specific phenotypes such as aerobic glycolysis.



J. S. QUICK



S. BATRA

POSTER A9

Correlation between Taqman low-density array and oligonucleotide microarrays in measuring multidrug resistance gene expression levels in the NCI-60 cell lines

JOSIAH N. ORINA, Emory University School of Medicine

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

■ The development of multidrug resistance (MDR) to chemotherapy remains a major issue in the treatment of cancer. Resistance exists against every effective anticancer drug and can develop by numerous mechanisms, including increased drug efflux, decreased drug influx, activation of DNA repair mechanisms, activation of detoxifying systems, and evasion of drug-induced apoptosis. The availability of a reliable expression tool that can identify genes and mechanisms critical to the development of MDR could potentially improve chemotherapy by offering clinicians the ability to predict and circumvent resistance. However, to date, a reliable and accurate high-throughput diagnostic tool assessing MDR genes is lacking in the clinical setting. Taqman low-density array (TLDA) is a state-of-the-art customized high-throughput quantitative RT-PCR technology that has several advantages over current gene expression methods such as oligonucleotide microarrays. These advantages include high sensitivity, specificity, reproducibility, robustness, and efficiency. Moreover, unlike microarrays, TLDA does not require validation of data using an independent mRNA quantitation technique. Despite these benefits, there have been few reports addressing the correlation between results obtained by TLDA and microarrays. In this study, the expression levels of 48 ATP-binding cassette (ABC) transporter genes implicated in MDR mechanisms were assessed in NCI-60 cell lines by using TLDA and compared to expression levels obtained from Affymetrix microarrays. Total RNA was extracted from the cancer cell lines, and expression of the selected genes was measured using TLDA. TLDA expression levels were then compared to microarray data published by Shankavaram et al. (*Molecular Cancer Therapeutics*, 2007). Statistically significant correlations ($p < 0.05$) between TLDA and microarray expression levels were observed for 28/46 (61%) genes. Correlation between the two platforms was not statistically significant ($p > 0.05$) for 15/46 (39%) genes. Two of the genes had no available microarray data. These results indicate that correlations between Affymetrix microarrays and TLDA are predominantly strong. However, given the aforementioned advantages of TLDA vis-à-vis microarrays, TLDA represents a more valuable tool for predicting resistance in the clinical setting.



J. N. ORINA



B. PEREZ

POSTER A10

Raising the bar for genomic prediction: applying statistical methods to ensure that success is not due to chance

BRADFORD PEREZ, Duke University School of Medicine

Mentor: Anil Potti, M.D., Duke University School of Medicine

■ Using Bayesian binary regression, we have previously shown that it is possible to predict sensitivity to chemotherapeutic agents, using the NCI-60 panel of cell lines that were assayed for sensitivity to chemotherapeutic agents and corresponding mRNA gene expression values. Cell lines determined to be most sensitive and most resistant are used to generate a gene list that distinguishes sensitive and resistant cell lines. Importantly, applying this gene list and associated coefficients to patient tumor samples has been shown to identify patients who respond or do not respond to treatment with a given chemotherapeutic agent.

Generation of cell-type-specific predictors of chemosensitivity may be a more appropriate way to identify sensitive or resistant tumors and further dissect drug resistance mechanisms. We have assayed 40 lung cancer cell lines for their sensitivity to six commonly used chemotherapeutic agents (cisplatin, docetaxel, paclitaxel, gemcitabine, pemetrexed, vinorelbine). As with the generation of some previous predictors of chemosensitivity, distinctions of sensitive and resistant cell lines did not separate appropriately in Bayesian binary regression model leave-one-out cross validation. In an effort to generate a model most likely to be accurate in predicting patient tumor sensitivity, we removed samples to optimize success. Given the bias introduced in model development, independent validation distinct from cross validation is imperative and must be performed before hypothesizing that models meaningfully predict sensitivity to chemotherapeutic agents.

Methods to ensure that accuracy of independent validation is not due to chance will be applied. These methods take into account the number of samples being independently validated and the true incidence of the binary phenotype in that independent validation. Similar statistical methods, if proven to be robust, will be applied to all developed models to further assess their true clinical relevance.

POSTER B1

Diversity, evolution, and pathogenesis of HIV-1 subtype C: reverse-transcriptase sequence analysis and the selection of nevirapine resistance

SUDEB C. DALAI, Stanford University School of Medicine

Mentor: David Katzenstein, M.D., Stanford University School of Medicine

■ The extraordinary genetic diversity of HIV-1 may explain divergent epidemic patterns in different regions of the world. Subtype C HIV-1, which causes the majority of global infections, is phylogenetically distinct from other subtypes. Moreover, inter-subtype variation in drug susceptibilities may preclude effective antiretroviral treatment. This study investigates the relationship between viral evolution, selection of drug resistance, and the return of susceptible virus among subtype C-infected women in Zimbabwe, who were administered single-dose nevirapine (SDNVP) to prevent mother-to-child-transmission of HIV-1.

First, applying phylogenetics algorithms, we characterized HIV-1 *env* sequence diversity in consensus plasma to demonstrate an explosive subtype C epidemic in southern Africa. We also applied Bayesian Markov chain Monte Carlo analysis, under different models of population growth, to a longitudinal dataset of reverse-transcriptase sequences over a 15-year period in Zimbabwe. We were able to reconstruct a most recent common ancestor virus, estimate the introduction of infection in this region at 1973, and approximate the phylogeny and viral dynamics of the epidemic from its origins to the present. Second, we investigated in vivo dynamics of select drug-resistance mutations in NVP-exposed women, using extensive molecular cloning methods. We observed a complex mixture of mutations with rapid overgrowth of wild type by 24 weeks, demonstrating the capacity of nonresistant virus to overtake mutations emerging shortly after drug selection. We are now applying highly sensitive allele-specific PCR assays to describe fitness of the signature NVP-resistance mutation, K103N. Finally, we applied in vitro phenotyping to determine drug susceptibilities in viral isolates from treated individuals. We found notable differences in susceptibilities between subtypes B and C, suggesting effects of polymorphisms and mutation interactions. Clonal analysis will reveal known and possibly new drug resistance mutations. Further genotype/phenotype correlations, and examination of polymorphic regions of functional HIV-1 genes, may uncover differences that can guide strategies for treatment and drug and vaccine design.

POSTER B2

Mechanisms of innate immune protection from HIV-1 disease in South Africa

AMBROSE HON WAI WONG, Washington University School of Medicine

Mentors: Marcus Altfeld, M.D., Ph.D., Harvard Medical School, and William Carr, D.V.M., Ph.D., Harvard Medical School and Nelson Mandela School of Medicine

■ KwaZulu-Natal (KZN) is one of the regions most devastated by HIV and AIDS in sub-Saharan Africa, with a higher HIV sero-prevalence rate (20–44% in adults) than any other province in South Africa. Disease course in HIV-1 infection is established early, suggesting that the innate immune system, including natural killer (NK) cells, may play a critical role in providing protection and determining viral load set points. Recent studies suggest that several immune-related genes, including killer Ig-like receptors (KIRs) and HLA, contribute to slower HIV-1 disease progression to AIDS. KIRs are expressed on NK cells and modulate their antiviral immune responses. Our specific aim was to determine the role of NK cells in slowing HIV-1 disease progression in chronically infected South African adults. To test the hypothesis that particular NK cell subsets expressing KIRs mitigate HIV-1 pathogenesis, we compared NK cells in a South African cohort of 60 patients, including HIV– patients and HIV+ patients stratified by CD4 count (>500, 200–500, 50–200, <50) as a clinical marker of disease progression. We used 16-color flow cytometry to 1) quantify stimulatory and inhibitory KIR expression and 2) qualitatively compare three functionally distinct NK cell subsets as defined by their expression of CD56, CD16 (FcyRIII-receptor), and KIRs, including a dysfunctional subset (CD56^{neg}CD16^{pos}KIR^{pos}) expanded in HIV-1-infected individuals. We also compared in vitro NK cell killing responses as measured by expression of CD107a (LAMP-1), a marker for cytotoxic granule exocytosis. We found significant differences in KIR and CD107a expression between patients in the various stages of HIV-1 disease. Here, we provide new insights on the role of innate immunity in HIV-1 disease progression among indigenous South Africans.

MONDAY
ATRIUM



S. C. DALAI



A. H. W. WONG

POSTER B3

The role of caspase-7 in restricting *Legionella pneumophila* replication in macrophages

KYLE VIANI, University of Michigan Medical School

Mentor: Gabriel Nuñez, M.D., University of Michigan Medical School

■ *Legionella pneumophila* causes Legionnaires' disease, a human multisystem illness characterized by severe pneumonia. Critical to the pathogenesis of the disease is the replication of the bacterium inside macrophages. In contrast to human macrophages, the macrophages from most mouse strains restrict *Legionella* replication. Previous studies demonstrate that the NOD-like receptor Ipaf restricts intracellular growth of *Legionella* in murine macrophages through activation of caspase-1 in response to flagellin. However, the mechanism by which caspase-1 controls bacterial growth remains unknown. Restriction of *Legionella* replication occurs independently of IL-1 β and IL-18, two known caspase-1 targets.

In this study, we sought to elucidate the events downstream of caspase-1 activation that are required to restrict *Legionella* growth. A list of caspase-1 substrates was compiled from a proteome-wide COFRADIC analysis, screening amino acid sequences for potential cleavage sites, and literature review. Lysates of infected wild-type and caspase-1 KO macrophages were immunoblotted with antibodies to potential substrates to detect cleavage by caspase-1. We demonstrate that caspase-7 is cleaved by caspase-1 in response to *Legionella* infection. The cleavage also occurs when caspase-1 is activated by stimulation with lipopolysaccharide and ATP instead of *Legionella*. Studies are currently underway to determine if cleavage of caspase-7 is required to restrict *Legionella* growth. Intracellular growth of the bacteria will be measured in macrophages from wild-type, caspase-7 KO, caspase-1 KO, and Ipaf KO mice. To determine the role of caspase-7 in vivo, we will infect mice intratracheally and enumerate the number of pulmonary bacteria. These experiments will determine the significance of the caspase-1-mediated cleavage of caspase-7 in response to *Legionella* infection.



K. VIANI



A. NGUYEN

POSTER B4

Evolution of multidrug resistance in *Pseudomonas aeruginosa* clinical isolates

ANVY NGUYEN, University of California, San Francisco, School of Medicine

Mentors: Jeanine P. Wiener-Kronish, M.D., and Susan V. Lynch, Ph.D., University of California, San Francisco, School of Medicine

■ *Pseudomonas aeruginosa* has the mechanisms for resistance to every clinically available class of antibiotics. Overexpression of Mex multidrug efflux pumps can lead to lowering of effective intracellular antibiotic concentrations, thereby conferring resistance to these drugs. A patient presenting with a highly virulent strain of *P. aeruginosa* acquired in Ghana, Africa, was admitted to the ICU at the UCSF Medical Center. Over the course of his stay, the infecting *P. aeruginosa* strain became increasingly multidrug resistant, and this resistance was tracked in real time. A random amplification of polymorphic DNA (RAPD) analysis was conducted. Assays for alginate, pyocyanin, and pyoverdinin production were performed. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to quantify MexB and MexF expression. Gene expression analysis was carried out using the Affymetrix GeneChip.

RAPD analysis performed on sequential cultures showed no differences, indicating the same strain throughout the patient's hospitalization. There were no changes in production of alginate, pyocyanin, and pyoverdinin between samples. qRT-PCR showed no change in transcription levels of MexF between samples. However, there was a steady increase in transcription levels of MexB, a gene encoding a component of the MexAB-OprM pump, over time. Gene expression analysis is currently underway.

There is increasing evidence that multidrug-resistant pumps play an important role in bacterial pathogenicity. Measurement of RNA transcript abundance of Mex pumps in sequential cultures from this patient showed increased expression of MexAB-OprM. Gene expression analysis should further elucidate the mechanisms of resistance in these *P. aeruginosa* clinical isolates.

POSTER B5

Transforming growth factor- β signaling abnormalities in central nervous system inflammatory diseases

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

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

■ Transforming growth factor- β (TGF- β) is a potent cytokine with widespread immunomodulatory effects. In vivo evidence suggests TGF- β signaling in T cells plays a key role in suppressing autoimmune reactions. Recent studies have demonstrated that TGF- β is critical for the induction of T-regulatory (Treg) cell differentiation. Tregs suppress the activation of immune cells to maintain immune system homeostasis and self-tolerance, and have been shown to be dysregulated in many autoimmune conditions, including multiple sclerosis (MS), myasthenia gravis, and type 1 diabetes.

TGF- β signal transduction occurs through the Smad signaling cascade; upon binding of the ligand to the receptor, the Smad2/3 heterodimer is phosphorylated, allowing it to complex with Smad4. The Smad2/3/4 complex translocates into the nucleus where it regulates transcription. One of the genes induced by TGF- β is Smad7, which then feeds back to modulate further signaling. In the autoimmune diseases irritable bowel syndrome and scleroderma, Smad7 expression levels are aberrant, indicating a perturbation in the TGF- β pathway. Given the evidence linking defective TGF- β signaling and autoimmunity, we sought to evaluate this pathway in subsets of peripheral blood mononuclear cells (PBMCs) isolated from MS patients. Specifically, mRNA expression levels of the proteins in the TGF- β signal transduction pathway were assessed using TaqMan quantitative real-time PCR.

Our findings showed that in PBMCs from normal donors and MS patients, there are statistically significant differences in mRNA expression levels for three of the proteins in the Smad pathway. Smad7 was downregulated, while Smad4 and TGF- β RII were upregulated. Smad7 reduction was demonstrated to be in the CD4+ T cell subset.

These preliminary results indicate a defect in TGF- β signaling in PBMCs from MS patients. This raises the possibility that defective TGF- β signaling results in dysfunctional Tregs that are unable to modulate the activation of other immune cells, leading to the neuroinflammation characteristic of MS.

POSTER B6

Effect of mammalian target of rapamycin inhibition on CD4+CD25^{high}FOXP3+ regulatory T cells compared with conventional CD4+ T cells

ELIZABETH A. ZAMBRICKI, Stanford University School of Medicine

Mentor: Robert Negrin, M.D., Stanford University School of Medicine

■ Allogeneic hematopoietic cell transplantation (aHCT) is currently utilized for treatment of patients with autoimmune diseases, organ transplantation, and hematopoietic malignancies. There are several beneficial effects of aHCT, including the graft-versus-tumor (GvT) reaction, which is capable of destroying residual tumor cells. However, aHCT is also imperfect due to the immunologic recognition and destruction of host tissues, graft-versus-host disease (GVHD). A major concept of research in this area has been to isolate populations of T cells that have specificity for tumor cells over host cell populations. Recent studies have identified CD4+CD25^{high}FOXP3+ regulatory T cells (T_{reg}), which are capable of suppressing lethal GVHD while maintaining beneficial GvT effects. One crucial condition of T_{reg} function and expansion in GVHD is calcineurin-dependent IL-2 production, which is hampered by the immunosuppressant cyclosporine A (CSA). In contrast to CSA, rapamycin (RAPA) inhibits the mammalian target of the rapamycin (mTOR) pathway. We and others have shown that RAPA, but not CSA, allows for T_{reg} expansion and function in humans and mice. Our studies herein found that T_{reg} and RAPA act synergistically in vivo, reducing conventional T cell (T_{conv}) expansion more effectively than when either treatment was used alone. Mechanistically, we found that T_{conv} and T_{reg} activation led to distinct pathway phosphorylation patterns. Namely, activated T_{reg} characteristically employ the signal transducer and activator of transcription 5 (STAT5) pathway, whereas T_{conv} typically utilize the phosphatidylinositol 3-kinase (PI3)/Akt/mTOR pathway in mouse models. Currently, our studies on human peripheral blood mononuclear cells (PBMC) hope to decipher if this discrete pathway signaling also applies to human cells. Preferential use of the mTOR pathway in T_{conv} as compared to T_{reg} elucidates the synergistic effect of RAPA and T_{reg} in GVHD defense as well as suggests that administration of RAPA as opposed to other immunosuppressive agents might prove beneficial when T_{reg} therapies are considered.



E. M. MEOLI



E. A. ZAMBRICKI

POSTER B7

Increasing expression or function of CXCR4, the receptor for SDF-1, enhances hematopoietic stem cell homing and engraftment

KRISTIN MARIE BERG, Creighton University School of Medicine

Preceptor: Harry L. Malech, M.D., National Institute of Allergy and Infectious Disease, National Institutes of Health

■ Hematopoietic stem cell (HSC) transplant is affected by host-donor matching, number of cells infused, and efficiency of homing to bone marrow (BM). Of factors reported to regulate homing to BM there is a major effect from interactions between stromal-cell derived factor-1 (SDF-1) and cognate cell surface receptor, CXC chemokine receptor-4 (CXCR4). Engraftment is improved by increasing expression of CXCR4 or by increasing the production or decreasing degradation of SDF-1 in BM. Our lab and others showed that treatment with protease inhibitors that prevent degradation of SDF-1 will enhance engraftment. Furthermore, our lab also showed that a gene transfer-mediated permanent increase in CXCR4 on HSC enhances engraftment. We have constructed and used non-integrating (N-I)-lentivector encoding CXCR4 to show that only a transient increase in CXCR4 in transduced HSC at time of transplant is required to enhance engraftment. C-terminal truncated mutant (mut-)CXCR4 has enhanced activity because the C-terminus is the target of down regulatory inhibition by G-protein receptor kinase-3 (GRK-3)-mediated phosphorylation and recruitment of β -arrestin. We conducted experiments showing that transduction of HSC with N-I-lentivector expressing mut-CXCR4 greatly enhances engraftment. Based on these studies, we speculated that preventing C-terminal phosphorylation of wild-type CXCR4 by reducing intracellular GRK-3 might also enhance activity of CXCR4 and enhance engraftment. We plan to target intracellular GRK-3 mRNA for degradation using RNA interference technologies in the form of electroporation delivery of siRNA or transduction with N-I-lentivectors encoding shRNA. We have shown that KG1, a CD34⁺ immortalized human HSC line, expresses functional CXCR4. We will target GRK-3 in KG1 and in primary human CD34⁺ HSC with siRNA or shRNA. The siRNAs have been designed and N-I-lentivectors encoding candidate shRNAs have been constructed. Increasing the efficacy of stem cell transplants has wide-reaching implications and is currently a vibrant area of study.



K. M. BERG



H. GARBER

POSTER B8

Targeting survivin as an antigen for development of a pediatric tumor mouse model

HAVEN GARBER, The Ohio State University College of Medicine

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

■ Murine models can serve as useful tools to study the interface between host immunity and cancer. The field of tumor immunotherapy has especially benefited from T cell receptor (TCR) transgenic mice, which generate T cells expressing a specific TCR that recognizes a tumor antigen. There is a need for such robust models in pediatric oncology, and to this end, we selected an antigen common to many childhood tumors. Survivin, an inhibitor of apoptosis protein, was selected due to its wide expressivity in pediatric tumors such as rhabdomyosarcoma and neuroblastoma. Survivin is an attractive target in immunotherapy because of its differential expression in cancer and its role in critical pathways required for tumor maintenance. Many studies have reported the generation of cytotoxic T lymphocytes (CTLs) specific for survivin in both mice and humans, and we sought to identify a vaccine that could elicit a reliable CTL response in the C57BL/6 mouse in hopes of selecting a T cell and TCR for use in a transgenic model.

A vaccine composed of one epitope from murine survivin (Surv₂₀₋₂₈) and a helper peptide from the hepatitis B viral core protein (HBV_{c128-140}) emulsified in incomplete Freund's adjuvant (IFA) elicited a CTL response with a frequency of 1 in 675 responding CD8⁺ T cells by ELISPOT. After 7 days of restimulation *in vitro*, splenocytes from vaccinated animals were cocultured with Surv₂₀₋₂₈ or irrelevant peptide. IFN- γ production by ELISA measured over 50,000 pg/ml for cells cultured with Surv₂₀₋₂₈ compared to 5,000 pg/ml for irrelevant peptide. CTL clones were then generated using limiting dilution cloning.

Future studies will be conducted to characterize the survivin-specific clones with regard to their ability to recognize pediatric tumors and their functional avidity. The T cell receptor from promising clones will be amplified and sequenced in preparation for development of a survivin-specific TCR-transgenic mouse.

POSTER B9

Investigation of the use of myxoma virus as oncolytic therapy for human melanoma

TANVI PARIKH, Weill Cornell Medical College

Mentors: Liang Deng, M.D., Ph.D., and Stewart Shuman, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center

■ Melanoma, a malignant neoplasm of melanocytes, is the most deadly form of skin cancer. Although thin melanomas can be treated adequately with local excision, patients with advanced melanoma have very poor prognoses. My research focuses on the use of myxoma virus as oncolytic therapy for human melanoma. Myxoma virus, a rabbit-specific poxvirus that belongs to the *Leporipoxvirus* genus, has been investigated as an oncolytic virus. Although myxoma virus has a narrow species specificity, it exhibits a tropism for human cancer cells. It replicates in human cancer cell lines that have increased levels of endogenous phosphorylated AKT (pAKT), a key signaling molecule implicated in oncogenesis. AKT has two phosphorylation sites, Thr-308 and Ser-473. Phosphorylation of both Thr-308 and Ser-473 is required for AKT activation. In this study, I tested myxoma virus replication in five human melanoma cell lines and B16 mouse melanoma. The results indicate that myxoma virus can replicate in each of the cell lines, with an increase of viral titers ranging from 40- to 100-fold during a 48-h infection period. There were variable endogenous levels of pAKT (Thr-308 and Ser-473) in each of the melanoma cell lines. Myxoma virus infection induced phosphorylation of AKT at both the Ser-473 and Thr-308 in some cell lines. Myxoma infection induced early apoptosis and cell death in the cells. Preliminary studies of intratumor injection of GFP-expressing myxoma virus demonstrated that myxoma virus infection was restricted to the tumor site and caused tumor cell necrosis. Our results demonstrate that myxoma replicates in human melanoma cells and induces apoptosis and cell death. Myxoma infection induces phosphorylation of AKT at critical residues, which may contribute to the viral tropism in these cells. Further studies using AKT inhibitors or siRNA knockdown of AKT might help to elucidate the precise mechanism.

POSTER B10

Evaluating the function of p53 mutants and prospective cancer treatments in single cells

IRUN BHAN, Harvard Medical School

Mentor: Galit Lahav, Ph.D., Harvard Medical School

■ The tumor suppressor protein p53 plays a central role in orchestrating cellular responses to DNA damage. In response to damage, this transcription factor activates the expression of multiple targets, including the cell cycle inhibitor p21 and the apoptosis inducer PUMA. The medical relevance of p53 is demonstrated by the fact that it is the protein most frequently inactivated during carcinogenesis. As cancer therapy typically functions by inducing DNA damage, treatment outcome is dependent on the status of p53 signaling. My research focuses on 1) the effect of common p53 mutations on p53 protein levels and function and 2) the ability of pharmacological agents to restore p53 function.

To study p53 function, I created a system that reports p53 levels as well as *p21* and *PUMA* promoter activity. H1299 cells, human lung carcinoma cells lacking *p53*, were transfected with constructs expressing different fluorescent proteins under the control of the endogenous *p21* and *PUMA* promoters. These cells were infected with lentiviruses expressing fluorescently tagged p53 variants (wild-type p53 or one of two common mutants). Cell cycle arrest, apoptosis, and the dynamics of these fluorescent markers will be studied using time-lapse microscopy in cells challenged with DNA damage. Prospective p53-stabilizing drugs will then be assessed for the ability to restore normal p53 function.

These experiments will provide novel, quantitative, single-cell data regarding the function of p53 mutants and may offer a new approach for defining groups of mutations that are susceptible to particular treatments. Further investigation may allow for treatment tailored to specific tumor genotype.



T. PARIKH



I. BHAN

POSTER B11

Transcriptional profiling of abdominal aortic aneurysms for disease-specific proteins

MARK HSU, Stanford University School of Medicine

Mentor: Philip S. Tsao, Ph.D., Stanford University School of Medicine

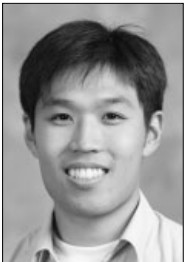


M. HSU

■ Abdominal aortic aneurysm (AAA) is a pathological thinning of the infrarenal aorta often accompanied by atherosclerosis, inflammation, and thrombosis. The underlying mechanism of AAA formation is poorly understood, with inadequate means to rapidly stratify an individual's risk of aneurysm development, progression, or rupture. We hypothesize that AAA has characteristic gene expression profiles that exhibit spatiotemporal inflammation, apoptosis, extracellular matrix breakdown, biomechanical forces, and thrombosis. AAA development in a mouse model was studied by infusing angiotensin II (1000 mg/kg) via subcutaneous osmotic pumps into 8- to 12-week-old mice on ApoE-KO background. Ultrasound was used to monitor AAA diameter at 0, 7, 14, and 28 days. AAA samples and control samples were collected at these time points for RNA isolation. RNA quality was validated by preliminary RT-PCR of select genes and processed for microarray. We also hypothesized that exercise decreases AAA growth by increasing laminar flow and inducing antioxidative and anti-inflammatory gene expression. Mice were run at 12 m/min on a treadmill for four weeks after implantation and monitored by ultrasound.

AAA diameter expands in a time-dependent manner. Average baseline diameter was 1.20 mm, expanding to 1.52 mm (n=29) at day 7, 1.63 mm (n=18) at day 14, and 1.71 mm (n=5) at day 28. Thirty-five percent of mice have findings resembling aortic dissection. RT-PCR revealed that 28-day AAA samples had 2.63-fold MMP-9 upregulation and 4.16-fold MCP-1 upregulation. Larger AAA diameters correlated with increased expression of these genes. Microarray analysis is pending. Initial results of exercise indicated an initial decrease in AAA growth (1.37 mm at day 7, 1.39 mm at day 14) before reverting to normal AAA growth by the third week.

The expansion of AAA diameter is related to the modulation of aortic wall gene expression, which will be further elucidated by microarray analysis. Exercise may have a beneficial effect in decreasing the rate of initial AAA expansion.



I. C. HAN

POSTER B12

The role of Bcl-x_L in retinal pigment epithelial cell survival in mouse models of age-related macular degeneration

IAN CARLOS HAN, Duke University School of Medicine

Mentor: Glenn J. Jaffe, M.D., Duke University School of Medicine

■ Retinal pigment epithelial (RPE) cell viability is a key factor in the pathogenesis of age-related macular degeneration. Bcl-x_L, a highly expressed anti-apoptotic factor in the Bcl-2 family, may play an important role in RPE cell survival. Previous work in our lab has shown that reducing Bcl-x_L expression in cultured human RPE cells causes apoptosis and that this effect is enhanced with exposure to subthreshold oxidative stress. To further elucidate the role of Bcl-x_L in RPE cell survival, we examined the effects of Bcl-x_L blockade in cultured mouse RPE cells and an in vivo mouse model. Wild-type mouse RPE cells were cultured and transfected with either Bcl-x_L-specific antisense oligonucleotide (ASO), a splice-switching (SS) ASO that converts Bcl-x_L mRNA transcripts to a pro-apoptotic variant, or a mismatched control. Subretinal injections were used to deliver ASOs into wild-type mouse eyes in vivo. Bcl-x_L expression and degree of RPE cell death were then assessed and compared between treatment groups.

Preliminary results suggest that in the absence of oxidative stress, mouse RPE cells may be resistant to apoptosis despite Bcl-x_L blockade. In human RPE cells, decreased Bcl-x_L expression with specific or SS ASOs resulted in significantly decreased viability compared to controls. The same concentration of these ASOs, however, does not appear to cause cell death in cultured mouse RPE cells. Similarly, RPE cell apoptosis in vivo does not appear to be increased following subretinal injections of low-dose specific or SS ASOs when compared with control groups.

Experiments involving increased ASO concentrations and exposure to oxidative stress will help clarify the importance of Bcl-x_L in mouse RPE cell survival. Other pro-apoptotic and anti-apoptotic factors may also contribute significantly to RPE cell viability, and characterizing their expression in mouse eye tissues may identify other potential key players in this dynamic balance between RPE cell death and survival.

8:30 A.M.

Aquaporin-2 trafficking: identifying binding partners using mass spectrometry as a hypothesis-generating approach**NICHOLAS A. ZWANG**, Harvard Medical School

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

■ Aquaporin-2 (AQP2) bears responsibility for concentrating urine in the kidney's inner medulla. The hormone vasopressin is the key signal that initiates translocation of AQP2-containing vesicles from the cytoplasm to the apical plasma membrane. Serine 256 phosphorylation on AQP2's C-terminal, cytoplasmic tail is a critical step for translocation. Recent studies have also identified three other phosphorylation sites at serines 261, 264, and 269. The cellular machinery to effect AQP2 trafficking, however, is poorly characterized. The aim of this study was to identify binding partners to phosphorylated sites of the AQP2 C-terminal tail by using mass spectrometry. We incubated cytosol isolated from rat inner medullary collecting ducts with "bait" peptides—representing the C-terminal AQP2 tail in its unphosphorylated, S256-phospho, and S261-phospho forms—to capture differentially bound proteins using a magnetic bead isolation technique. Bound proteins were eluted and subjected to mass spectrometric analysis to 1) establish their identities and 2) estimate their affinities for the differentially phosphorylated AQP2 tail peptides using label-free, quantitative LC-MS/MS.

Our studies have identified several proteins of interest. These include protein phosphatase 2A (a serine/threonine phosphatase), cysteine-rich protein 2 and cysteine-and-glycine-rich protein 1 (LIM domain proteins involved with actin cytoskeletal regulation), annexins A2 and A11 (calcium-dependent phospholipid binding proteins with putative roles in membrane trafficking), glutathione-S-transferase, and heat shock protein 70.

Each of these protein identifications generates a hypothesis regarding the regulation and mechanism of AQP2 trafficking. Our ongoing work aims to test these hypotheses by verifying the above interactions, quantifying their affinities for the differentially phosphorylated C-terminal tail of AQP2, and localizing interactions at the subcellular level.

8:45 A.M.

Nitric oxide stimulates inducible cAMP early repressor (ICER) gene expression in rat pulmonary smooth muscle cells via a Ca²⁺-dependent pathway**KIM JIRAMONGKOLCHAI**, Vanderbilt University School of Medicine

Mentor: Kenneth D. Bloch, M.D., Massachusetts General Hospital and Harvard Medical School

■ **Introduction:** Many human vascular diseases, including hypertension, atherosclerosis, septic shock, and diabetes mellitus, are linked to abnormalities in nitric oxide (NO) synthesis. NO regulates crucial cellular activities in vascular smooth muscle cells at least in part via changes in gene expression. Previously, we characterized the transcriptional profile of rat pulmonary artery smooth muscle cells (RPaSMC) treated with S-nitroso glutathione (GSNO), a NO-donor, and identified a cohort of genes whose expression was induced by GSNO via activation of cAMP-dependent protein kinase (PKA) and the transcription factor cAMP-responsive element binding protein (CREB). Exposure of RPaSMC to NO rapidly and dramatically increased gene expression and protein levels of inducible cAMP early repressor (ICER), a negative regulator of CREB. We aimed to characterize the mechanisms by which NO regulates ICER gene expression.

Methods and results: ICER mRNA levels were first measured in RPaSMC pretreated with BAPTA/AM, an intracellular Ca²⁺ chelator, followed by exposure to GSNO. BAPTA/AM was shown to block the ability of GSNO to induce ICER gene expression, suggesting that ICER expression by NO is Ca²⁺-dependent. To determine the Ca²⁺ source, we measured ICER mRNA levels in RPaSMC pretreated with nifedipine, a voltage-dependent Ca²⁺ channel inhibitor; SKF-96365, a store-operated calcium channel (SOCC) inhibitor; or ryanodine, an inhibitor of the ryanodine Ca²⁺ channel. We found that ryanodine and SKF-96365, but not nifedipine, decreased the ability of NO to induce ICER. These results suggest that NO stimulates cytosolic Ca²⁺ entry via SOCC and ryanodine Ca²⁺ channels. In addition, MEK1 inhibitor U0126 was shown to block the ability of NO to increase ICER mRNA levels, indicating that MAP kinase plays a role in ICER expression.

Conclusion: These findings suggest that the regulation of ICER by NO is governed by cAMP, PKA, Ca²⁺, and MAP kinase-dependent signaling. Ongoing studies are directed toward elucidating the sequential signaling events by which NO regulates gene expression in vascular smooth muscles.

TUESDAY
ROOM A

N. A. ZWANG



K. JIRAMONGKOLCHAI

TUESDAY
ROOM A

9:00 A.M.

Characterization of primary and restenotic atherosclerotic plaque from the superficial femoral artery: differential expression of the TGF- β signaling protein, Smad3**RACHEL S. EDLIN**, New York University School of Medicine

Mentor: K. Craig Kent, M.D., New York Presbyterian Hospital and Weill Cornell Medical College

■ The growing use of endovascular atherectomy has provided the opportunity to elucidate the pathophysiology of restenotic plaque. Our previous work in an animal model of restenosis reveals that Smad3, a major TGF- β signaling molecule, is upregulated in injured arteries. We therefore hypothesized that differential expression of Smad3 in human restenosis versus primary plaque might account for the enhanced smooth muscle cell (SMC) proliferation found in restenotic lesions.

Immunohistochemical studies were performed on specimens retrieved from the superficial femoral artery of patients undergoing atherectomy for primary atherosclerotic lesions (n=5) or restenotic lesions (n=3). Analysis of restenotic lesions revealed a significantly higher expression of α -actin ($78.37\% \pm 2.16\%$ vs. $54.22\% \pm 2.17\%$, $p=0.0003$), PCNA ($66.46\% \pm 0.73\%$ vs. $24.44\% \pm 1.56\%$, $p=0.001$), and Smad3 ($25.87\% \pm 1.48\%$ vs. $0.00\% \pm 0\%$, $p=0.0001$) while primary lesions expressed significantly more CD45 and CD68. Further studies demonstrated colocalization of Smad3 with PCNA as well as with α -actin, thus suggesting a role for Smad3 in SMC proliferation. Using an *in vitro* tritiated thymidine incorporation assay, we found that although TGF- β inhibited rat SMC proliferation, in the setting of Smad3 over-expression via adenovirus-mediated gene transfer, the inhibitory effect of TGF- β was reversed. Furthermore, downregulation of Smad3 using a siRNA to Smad3 significantly decreased basal cell proliferation in comparison to control siRNA.

In conclusion, we have confirmed in humans that there is significantly more cell proliferation in restenotic plaque compared to primary atheromas. Additionally, restenotic plaque is comprised mainly of SMCs whereas primary atheromas contain significantly more inflammatory cells. These differences in cellular composition and cell proliferation in conjunction with the finding that Smad3 is expressed exclusively in restenotic disease suggest that TGF- β through Smad3 signaling may play an essential role in the distinct pathophysiology underlying restenosis.



R. S. EDLIN



C. KIM

9:15 A.M.

Prevention of corneal neovascularization *in vivo* by transplantation of endostatin-expressing corneal epithelial cells via silk biomatrix**CHARLES KIM**, University of California, Davis, School of Medicine

Mentor: Mark I. Rosenblatt, M.D., Ph.D., Weill Cornell Medical College

■ Angiogenesis is a dynamic process regulated by a balance of pro- and anti-angiogenic factors. In an uninjured state, the cornea is avascular, as anti-angiogenic factors outweigh pro-angiogenic factors to inhibit blood vessel formation. However, a variety of corneal insults can lead to neovascularization, producing subsequent vision loss and blindness. Current treatment modalities nonspecifically target a wide array of biologic processes involved in angiogenesis and have largely proven to be ineffective. In this study, we developed a lentiviral vector capable of transferring an anti-angiogenic gene to corneal epithelial cells. Purified populations of transduced cells were isolated, with the hope of delivering these cells via silk biomatrix to injured rabbit corneas and inhibiting angiogenesis.

We have designed a lentiviral vector system containing the gene for endostatin, a 20-kD protein derived from the C-terminus of collagen XVIII that has shown marked anti-angiogenic activity. The vector also contains a GFP-reporter gene as well as an I κ k linker sequence, which allows endostatin to be secreted from cells. Following transduction of rabbit corneal epithelial cells with our vector, the cells were sorted based on GFP expression. Quantitative PCR verified the upregulation of RNA specific to endostatin in transduced cells, with higher RNA levels seen in sorted versus unsorted cells. In addition, Western blotting using supernatant from transduced cells confirmed the secretion of endostatin from these cells.

Lentiviruses can infect corneal epithelial cells in an efficient and stable manner, causing an increase in specific RNA and protein expression in transduced cells. Preliminary studies suggest that endostatin produced by our transduced cells inhibits the growth of human umbilical vein endothelial cells, reflecting its anti-angiogenic activity *in vitro*. Future studies will be performed to confirm these findings and to deliver these cells to rabbits following corneal injury to demonstrate their efficacy *in vivo*.

9:30 A.M.

Melatonin suppresses peripheral *pro-opiomelanocortin* expression: a proposed mechanism of seasonal affective disorder

JOSHUA SCHULMAN, Harvard Medical School

Mentor: David E. Fisher, M.D., Ph.D., Massachusetts General Hospital and Harvard Medical School

■ The suntanning response occurs when ultraviolet (UV)-mediated DNA damage stabilizes p53 in keratinocytes, which in turn stimulates the *pro-opiomelanocortin* (*POMC*) promoter. The *POMC* gene product is cleaved into several bioactive peptides, including α -melanocyte-stimulating hormone, which activates pigment production in melanocytes, and β -endorphin. Because β -endorphin can promote positive mood, UV-induced β -endorphin expression has been suggested to reinforce sun-seeking behavior. Interestingly, among people who visit indoor tanning salons most frequently, the prevalence of seasonal affective disorder (SAD) is highly overrepresented, suggesting a possible self-medicating role for cutaneous UV exposure. It is believed that pineal melatonin secretion underlies the pathophysiology of SAD, though a direct role for melatonin in modulating mood has not previously been demonstrated. Our study tests the hypothesis that melatonin contributes to depressive symptoms by decreasing peripheral *POMC* expression.

Using cultured primary human keratinocytes, we have found that physiologic concentrations of melatonin suppress *POMC* mRNA levels. *POMC* suppression by melatonin occurs via a cAMP-mediated pathway, which can be blocked by preadministration of a melatonin receptor antagonist, and which can be rescued by treatment with forskolin, an adenylate cyclase activator. Furthermore, the suppression of *POMC* by melatonin can be rescued by subsequent UV exposure.

In total, these results suggest that the mechanism of SAD may involve suppression of peripheral *POMC* by melatonin, and frequent tanning among SAD patients may rescue *POMC* expression in the skin.

9:45 A.M.

Fluorescence-enhanced imaging as an aid to the surgical cytoreduction of peritoneal carcinomatosis of ovarian origin: evaluation in a murine model

MICHELLE RAE LONGMIRE, University of New Mexico School of Medicine

Preceptor: Peter L. Choyke, M.D., National Cancer Institute, National Institutes of Health

■ Epithelial ovarian cancer has the highest mortality rate among all gynecologic cancers. The majority of women with ovarian cancer have advanced-stage disease with diffuse peritoneal metastasis at the time of diagnosis, resulting in poor overall prognosis. Hope is offered, however, by the fact that ovarian cancer is unique among solid tumor cancers for prognosis being greatly affected by resection of metastatic disease. Surgical cytoreduction is one of the most effective interventions to improve patient outcomes. However, the small size of tumor implants and poor visual contrast between tumor and nontumor tissue complicate detection and removal of metastatic disease and therefore pose a limitation in the attainment of maximal benefit of surgical therapy. It has thus been proposed that surgical cytoreduction could be greatly enhanced with the use of tumor-specific optical fluorophores that serve as an intraoperative guide to tumor locations. With this in mind, we have developed a novel surgical method for the identification and removal of ovarian cancer metastases, using cancer-specific optical probes and a fluorescence camera that displays image data in real time. Assessment of this technology in a murine model of disseminated ovarian cancer (n=6) revealed that use of fluorescence-enhanced image guided surgery (FEIGS) after reduction using standard surgical methods substantially increased removal of tumor burden. Quantity of tumor removed was increased by an average of 81%, and total amount of tumor removed was more than doubled in several of the models evaluated. Percent increase in quantity of tumor removed ranged from 28 to 158% and lesions as small as 100 microns could be identified and removed. These results suggest that intraoperative fluorescence imaging offers a potentially powerful adjunct to standard methods for the surgical cytoreduction of peritoneal carcinomatosis of ovarian origin.



J. SCHULMAN



M. R. LONGMIRE

TUESDAY
ROOM A

10:00 A.M.

Autocrine function of fibroblast growth factor 23 in bone mineralization and osteoblast differentiation in vitro

SOMI KIM, Harvard School of Dental Medicine

Mentor: Beate Lanske, Ph.D., Harvard School of Dental Medicine



S. KIM

■ Fibroblast growth factor 23 (FGF-23) has been identified as a major phosphate-regulating hormone. Changes in FGF-23 levels have been found to be responsible for various human diseases, including autosomal dominant hypophosphatemic rickets, oncogenic osteomalacia, X-linked hypophosphatemia, autosomal recessive hypophosphatemia, chronic kidney disease, and familial tumoral calcinosis. Studies using a number of mouse models resembling these diseases have confirmed the importance of FGF-23 in bone mineralization, phosphate homeostasis, and vitamin D metabolism; however, the molecular mechanism of FGF-23's regulation is not yet completely understood. FGF-23's direct effects on bone mineralization have been especially difficult to establish. Complete lack of Fgf-23 (*Fgf-23*^{-/-}) exhibits hyperphosphatemia, whereas overexpression of FGF-23 (*FGF-23 TG*) exhibits hypophosphatemia.

However, both mutants result in similar bone abnormalities such as osteomalacia.

In order to investigate the role of FGF-23 on skeletal mineralization and bone cell differentiation independent of the serum phosphate level, we performed in vitro experiments using primary osteoblast cultures from 3- to 5-day-old *Fgf-23*^{-/-} and *FGF-23 TG* mouse calvaria. We analyzed osteoprogenitor proliferation, osteoid nodule formation, mineralization, and gene expression at various time points. No significant difference in proliferation was detected in both mutant genotypes when compared to wild-type littermates. In contrast, cultures from *Fgf23*^{-/-} showed significantly less mineralization whereas *FGF-23 TG* showed significantly more mineralization than wild-type, suggesting a local effect of FGF23 on bone cells. Moreover, *Fgf-23*^{-/-} had significantly decreased calcium deposition at 14, 21, and 28 days of culture, and *FGF-23 TG* had significantly increased calcium deposition at 7 and 14 days of culture when compared to wild type.

These data suggest that FGF-23 exhibits local effects on bone mineralization that are independent of its systemic effects on phosphate homeostasis. The mechanisms responsible for these effects still remain to be elucidated.

8:30 A.M.

Investigating the effects of Tau aggregates on primary neurons**AMIE YOO-YOUN LEE**, University of California, San Francisco, School of Medicine

Mentor: Marc I. Diamond, M.D., University of California, San Francisco

■ The microtubule-associated protein Tau normally functions to promote tubule assembly and stabilization in neurons. In neurodegenerative disease, however, Tau accumulates to form insoluble fibrillar aggregates. These conditions, collectively known as tauopathies, encompass a wide range of diseases, including Alzheimer disease, progressive supranuclear palsy, and frontotemporal dementia. Prior work in our lab has demonstrated that extracellular Tau aggregates are capable of entering cultured cell lines and are subsequently able to induce fibrillization of intracellular Tau.

Here, I explored the effects of Tau aggregates on primary neuron cultures. I first purified recombinant Tau microtubule-binding-region (MTBR), the known functional and pathogenic region of the Tau protein. I induced oligomerization of the purified Tau with aracidonic acid and confirmed the formation of oligomers by atomic force microscopy. I found that extracellular Tau oligomers were robustly taken up by rat primary neurons. In contrast, Tau monomers were incapable of entering neurons. Exposure to Tau oligomers also appeared to induce distinct morphologic alterations in primary neurons, primarily beading or thickening of axons and enlargement of cell bodies, though the significance of this change is unclear. To investigate the effects of Tau oligomers on intracellular Tau, I electroporated neurons with a tau-yfp construct. Exposure of the electroporated neurons to extracellular Tau oligomers resulted in increased levels of insoluble Tau-Yfp, suggesting that oligomers might trigger intracellular Tau aggregate formation. Of interest, very preliminary experiments with organotypic hippocampal slice cultures have demonstrated possible cell-to-cell spread of Tau aggregates via intraneuronal Schaffer collateral connections.

Together, these data indicate that Tau aggregates are capable of entering primary cultured neurons and this can potentially seed aggregation of intracellular Tau. Further advances in our understanding of Tau and its effect on neurons are necessary to develop more effective therapeutic strategies for many incurable neurodegenerative diseases.

8:45 A.M.

Stability and degradation of the survival of motor neuron protein**ERIC C. MUÑOZ**, Oregon Health and Science University School of Medicine

Preceptor: Kenneth H. Fischbeck, M.D., National Institute of Neurological Disorders and Stroke, National Institutes of Health

■ Patients with spinal muscular atrophy (SMA) have symmetrical proximal weakness of muscles. The severity of SMA varies. In the most common and severe category, SMA type I, patients are typically diagnosed in infancy and do not survive beyond two years of age. There is currently no effective treatment for SMA. SMA is caused by a deficiency in the survival of motor neuron (SMN) protein, and inhibiting the degradation of this protein could be a promising treatment strategy. Evidence indicates that the SMN protein is degraded via the ubiquitin proteasome system. The purpose of our study has been to investigate proteasome inhibition as a possible treatment for SMA.

HEK 293T cells and SMA patient-derived fibroblasts were treated with escalating doses of proteasome inhibitors, and the effects of these treatments on SMN protein levels were examined. Western blot analysis demonstrated that two of several novel proteasome inhibitors (Santhera Pharmaceuticals, Switzerland) and the FDA-approved proteasome inhibitor bortezomib produced a dose-related increase in SMN protein levels. Also, our preliminary *in vivo* studies indicate an increase in SMN protein levels in the muscle tissue of mice treated with a proteasome inhibitor.

Our results indicate that proteasome inhibition may be an effective treatment for SMA. We now plan to examine the effects of the proteasome inhibitors on survival and motor function *in vivo*. Additionally, as posttranslational modifications may regulate proteasomal degradation, we aim to further characterize modifications that may affect SMN protein stability.

TUESDAY
ROOM B

A. Y.-Y. LEE



E. C. MUÑOZ

TUESDAY
ROOM B

9:00 A.M.

Subventricular neural progenitor cells differentiate into oligodendrocytes after status epilepticus**CHARLES MIKELL**, Columbia University College of Physicians and Surgeons

Mentors: Guy McKhann, M.D., and James Goldman, M.D., Ph.D., Columbia University College of Physicians and Surgeons

■ Temporal lobe epilepsy (TLE) is the most common intractable seizure disorder. The most frequent pathology found in TLE is hippocampal sclerosis, which is characterized by focal neuron loss and astrogliosis. The origin of astrocytes in the areas of astrogliosis remains unclear. They differ from normal protoplasmic cells in that they are deficient in potassium channels and glutamate transporters, and therefore may contribute to recurrent seizures.

We hypothesized that a portion of these astrocytes might arise from the nearby subventricular zone (SVZ). To study the contribution of the SVZ to astrogliosis, we performed a fate-mapping study on the pilocarpine model of TLE in rats using stereotactic injection of a green fluorescent protein (GFP)-labeling retrovirus into the SVZ. After 1 day, we induced status epilepticus, and sacrificed animals on days 3, 7, 14, and 28 after seizures.

We found that status epilepticus markedly increases cellular proliferation in the subventricular zone, and a large number of cells were labeled in the corpus callosum, relative to controls. These cells differentiated predominantly into oligodendrocytes, though some NG2+ glia were also labeled. However, only a minimal number of astrocytes were labeled, and they were located within white matter surrounding the SVZ.

We concluded that status epilepticus causes differentiation of subventricular cycling progenitors mostly into cells of oligodendrocyte lineage, and the SVZ contribution to hippocampal astrogliosis is minimal. At the studied time points, newborn cells migrated only within white matter.



C. MIKELL



D. J. LEE

9:15 A.M.

The functional role of aquaporin-4 in epileptogenesis**DARRIN J. LEE**, University of California, Irvine, School of Medicine

Mentor: Devin K. Binder, M.D., Ph.D., University of California, Irvine

■ Aquaporin-4 (AQP4) is a transmembrane protein that regulates cellular water transport in the brain. In addition to water transport, AQP4 is hypothesized to modulate ion homeostasis, for example, with its molecular partner, the inwardly rectifying K⁺ channel Kir4.1. Together, the functions of AQP4 make it a likely candidate to regulate seizure onset and development. The aim of this study is to examine the role of AQP4 in epileptogenesis in the hippocampus using the systemic pilocarpine and intrahippocampal kainic acid mouse models of epilepsy.

After the induction of status epilepticus (SE), the AQP4 wild-type and null mice are monitored with continuous digital video and electroencephalography (EEG) recording for the development of spontaneous seizures. Immunohistochemistry is done at 1, 4, 7, and 30 days after the initial SE to determine changes in AQP4, Kir4.1, and other glial-specific proteins. In the pilocarpine model, spontaneous seizures develop between post-SE day 3 and 5. These consist of focal and/or generalized seizures (grades 1–3 in the Racine classification). Clear upregulation of AQP4 immunoreactivity is observed at 4 and 7 days, but this had largely returned to baseline by 30 days. Marked upregulation of glial fibrillary acidic protein (GFAP) is also observed at 4 and 7 days, indicating pathologic glial cell response to the episode of SE. Interestingly, in contrast to the upregulation of AQP4 and GFAP, transient downregulation of Kir4.1 is observed.

Our data suggest that AQP4 and its molecular partner Kir4.1 are indeed coordinately regulated following SE, and therefore suggest a potential glial contribution to epileptogenesis. Further electrophysiologic analysis of AQP4 wild-type versus AQP4-null mice will determine whether AQP4 contributes directly to altered epileptogenesis. Furthermore, identification of the glial cell proteins that are altered immediately prior to the development of epilepsy in these models provides an opportunity to identify novel glial targets for epilepsy therapy.

9:30 A.M.

Electrophysiology and functional magnetic resonance imaging during spike-wave seizures in WAG/Rij rats

DAMIEN J. ELLENS, Yale University School of Medicine

Mentor: Hal Blumenfeld, M.D., Ph.D., Yale University School of Medicine

■ Functional magnetic resonance imaging (fMRI) studies show increases and decreases in the blood oxygenation level dependent (BOLD) response in selective neuronal networks. An increase in the BOLD-fMRI response has been shown to correspond with increases in neuronal activity and cerebral blood flow (CBF) using laser Doppler flowmetry (LDF) in rat somatosensory cortex. However, decreases in BOLD-fMRI response are poorly understood. Wistar albino Glaxo rats of Rijswijk (WAG/Rij), an established model of human absence epilepsy, can be used to study the relationship between the hemodynamic response and underlying neuronal signals. Aims of this study were to map hemodynamic responses and to calculate neuro-energetics in the whole brain during spike-wave discharges (SWD) using multimodal fMRI, and to relate fMRI signals to electrophysiological changes during SWD.

BOLD-fMRI was performed at 9.4 Tesla with simultaneous EEG. fMRI signal increases were observed in the somatosensory and motor cortices, thalamus, and brainstem during SWD. Parallel electrophysiological and LDF recordings revealed increases in neuronal activity and CBF in the somatosensory cortex. Increases in CBV corresponded to the *increases* in BOLD signal. Interestingly, fMRI measurements also showed prominent *decreases* in BOLD response in the caudate-putamen and hippocampus during SWD. CBV *decreases* were in partial agreement with BOLD decreases.

Based on these results, BOLD-fMRI increases are associated with regional increases in neuronal activity, and increased CBV, and BOLD decreases are associated with decreased CBV. Previous work in tonic-clonic seizures showed *decreased* BOLD signals with *increased* CBV. These findings suggest that the physiological mechanisms underlying BOLD decreases may differ between seizure types. Further investigations are needed to determine if regions of BOLD decreases during SWD represent vascular steal, a primary neuronal mechanism, or a defect in neurovascular coupling. These findings contribute to the understanding of fMRI signal changes in human epilepsy imaging.

9:45 A.M.

Characterization of early murine cytomegalovirus infection in vivo

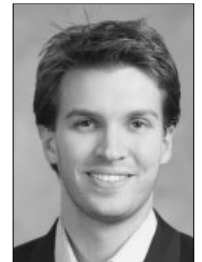
KIMBERLY HSU, Washington University School of Medicine

Mentor: Wayne Yokoyama, M.D., Howard Hughes Medical Institute, Washington University School of Medicine

■ Although the pathogenesis and immune control of murine cytomegalovirus (MCMV) has been increasingly elucidated over the past few years, few studies have addressed in vivo pathogenesis within the first day of infection, as most studies investigate infection after at least two days. Moreover, MCMV eventually spreads to infect many cell types, but it is unclear which cells are initially infected.

In this study, we observe the time course of MCMV infection within the first two days using a recombinant MCMV-EGFP, allowing for visualization of infected cells. Infected cells were detected in the spleen and liver as early as four hours postinfection (p.i.), with the infection becoming increasingly widespread by 48 hours p.i. Infection in the spleen followed a distinctive pattern, beginning in the marginal zone at 4–6 hours p.i. and spreading well into the red pulp by 24 hours p.i. Infection by intraperitoneal versus intravenous injection produced the same pattern of infection, suggesting that free virus traffics to the spleen to infect splenic cells. Infected cells in the spleen were identified as stromal cells by immunohistochemistry. To determine whether this pattern of infection was due to tropism of the virus or structural convenience, we infected mice with cowpox virus and the pattern of infection was compared to that of MCMV infection. Infection with cowpox virus produced a distinctly different pattern, with primary targets being macrophages.

Thus, although active long-term MCMV infection can appear in nearly any organ, we have shown that the virus has a specific tropism for splenic stromal cells and hepatocytes within the first day of infection. This early time period is important for the virus to establish an infection, and for the host to activate its immune system.



D. J. ELLENS



K. Hsu

TUESDAY
ROOM B

10:00 A.M.

Establishment of an immunosuppressive environment by cutaneous squamous cell carcinomas and its reversal by imiquimod

SUSAN JEN HUANG, Harvard Medical School

Mentors: Rachael A. Clark, M.D., Ph.D., Brigham and Women's Hospital, and Thomas S. Kupper, M.D., Harvard Medical School and Brigham and Women's Hospital

■ Organ transplant recipients on T cell immunosuppressant medications develop numerous and aggressive squamous cell carcinomas (SCCs) of the skin, suggesting that T cell function is critical to controlling SCC. By isolating T cells from SCCs, we previously showed that SCCs evade the immune response by at least two mechanisms. First, SCCs fail to express E-selectin on tumor vessels, thus excluding skin homing T cells, which are most capable of recognizing tumors. Nitric oxide (NO) is a known suppressor of endothelial E-selectin expression. Immunofluorescence studies on SCCs showed an accumulation of iNOS⁺ dendritic cells (DCs). We hypothesize that iNOS⁺ DCs produce NO, which suppresses E-selectin expression on tumor vessels. These iNOS⁺ DCs express the chemokine receptor CCR2 by immunostaining. SCCs also evade the immune system by recruiting regulatory T cells (Treg), cells that can suppress immune responses. In SCCs, FOXP3⁺ Treg accounted for 50% of T cells. Human glioblastomas recruit Treg into tumors via CCR2. We found that SCC Treg expressed CCR2 at 40-fold higher levels than normal skin Treg via microarray studies. Flow cytometry confirmed that the majority of FOXP3⁺ Treg in SCCs expressed CCR2, versus a minority of normal skin Treg. Microarray studies also showed increased production of CCR2 ligands MCP-2 and MCP-3 in SCCs. We thus hypothesize that CCR2 ligand expression by SCCs mediates iNOS⁺ DCs and Treg accumulation, thus establishing an immunosuppressive environment. Imiquimod, a topical immune modulator used to treat skin cancers, counters tumor immune evasion by indirectly upregulating tumor vessel E-selectin and decreasing the percentage and suppressive ability of Treg. Treg from treated SCCs produced less IL-10 and TGF-β. Although imiquimod is not used in transplant patients for fear of inciting transplant rejection, other drugs that can prevent or nullify the immunosuppressive environment, including CCR2 inhibitors, may prove promising in the treatment of these tumors.



S. J. HUANG



A. YONEKURA COLLIER

10:15 A.M.

Antigen presentation by lymph node stroma: potential for tolerogenic immunotherapy

AI-RIS YONEKURA COLLIER, Harvard Medical School

Mentor: Shannon J. Turley, Ph.D., Dana-Farber Cancer Institute and Harvard Medical School

■ Self-reactive T cells escaping central tolerance were thought to be regulated peripherally only by dendritic cells (DCs). We and others recently described a novel lymph node stroma cell (LNSC) capable of inducing antigen-specific deletional tolerance among CD8⁺ T cells. LNSCs mediate tolerance through expression of peripheral tissue antigens, similarly to medullary thymic epithelial cells. To characterize this rare lymph node cell, we developed a system to expand LNSCs *ex vivo* (LNSCex). LNSCex take up antigen for cross-presentation to CD8⁺ T cells, inducing proliferation. LNSCex express MHC class I and II and costimulatory molecules, enabling LNSCex to interface with and modulate T cell responses in an antigen-specific manner.

Transfer of antigen-loaded DCs has been used to induce CD8⁺ tolerance *in vivo*. DCs respond to inflammatory stimuli by upregulating MHC and costimulatory molecules, becoming potent immune activators. Thus, given the right microenvironment, they can potentially drive antigen-specific immune responses. Contrastingly, in response to inflammation, LNSCs downregulate a costimulatory molecule, CD80, and upregulate a coinhibitory molecule, PD-L1, thereby retaining a tolerogenic phenotype. Antigen-loaded LNSCs may prove safer and more effective as a tolerogenic vaccine than DCs. The nonobese diabetic (NOD) mouse model of spontaneously arising type-1 diabetes was used to test the efficacy of LNSCex in reinforcing tolerance *in vivo*. Diabetes incidence was monitored after transfer of insulin peptide-pulsed LNSCex. NOD mice given insulin peptide-pulsed LNSCex exhibited a significant delay in diabetes onset compared to PBS-injected controls.

Ex vivo expansion methods have facilitated characterization of a novel lymph node cell capable of inducing CD8⁺ tolerance. Current therapeutic approaches for autoimmunity and graft rejection work through global immunosuppression and cytokine blockade. We introduce a novel cell therapy that utilizes a physiological mechanism of tolerance to modulate autoimmunity in NOD mice. LNSCs have the potential for use as therapy in graft rejection or autoimmune disease.

TUESDAY
AUDITORIUM

8:30 A.M.

Methods toward understanding cell differentiation and self-renewal in hepatocellular carcinoma

FEI DONG, Case Western Reserve University School of Medicine

Preceptor: Xin Wei Wang, Ph.D., National Cancer Institute, National Institutes of Health

■ Hepatocellular carcinoma (HCC) is a heterogeneous disease. Gene expression profiles distinguish cancer subtypes within the patient population, and heterogeneous cell populations may coexist within any given cancer. Recently, our laboratory and others have identified subtypes of HCC, which correspond to the normal hepatic differentiation lineage by protein markers and gene expression. Specifically, HCC with features of hepatic progenitor cells has been defined by the expression of EpCAM and alpha-fetoprotein (AFP).

This project aims to utilize these stem cell markers to capture cellular differentiation and self-renewal by live imaging. A DNA plasmid was constructed containing the EpCAM promoter driving the expression of green fluorescence protein (GFP) and the AFP promoter driving the expression of red fluorescence protein (RFP). The HuH7 HCC line transfected with this plasmid expressed RFP, but GFP was not present, likely due to low endogenous activity of the EpCAM promoter. The generation of HuH7 clones with stable integration of this plasmid and their phenotype will be discussed.

8:45 A.M.

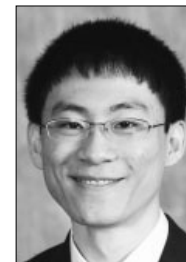
Combination therapy of radiofrequency ablation and bevacizumab in the treatment of ectopic hepatocellular carcinoma

ASHESH THAKER, University of California, Los Angeles, David Geffen School of Medicine at UCLA

Preceptor: Bradford Wood, M.D., Warren Grant Magnuson Clinical Center, National Institutes of Health

■ Radiofrequency ablation (RFA) is a minimally invasive treatment for localized primary and secondary solid tumors in various locations. Energy applied at the tip of a percutaneously inserted needle-electrode causes ionic agitation and frictional heat in the surrounding tissue. The heat associated with RFA induces local coagulation necrosis when temperatures reach 50–60°C. The efficacy of RFA is affected by blood vessels near the tumor, which can dissipate heat and limit the size of the necrosis zone. This is often a significant limitation when treating highly vascular or angiogenic tumors such as hepatocellular carcinoma (HCC) of the liver. The goal of the present study is to improve the efficacy of RFA by combining it with the antiangiogenic drug bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF). It has been previously shown that a systemic antiangiogenic agent can improve tumor ablation; however, the temporal relationship between applying antiangiogenic therapy and ablation is unknown.

In the first phase of the present study, athymic nude mice carrying human HCC xenografts were treated with a single 100-µg dose of bevacizumab and tumor blood flow was monitored daily with power Doppler ultrasound for one week. Preliminary results show a 30% decrease in tumor blood flow on day two of antiangiogenic therapy, suggesting a role for power Doppler ultrasound as a surrogate biomarker to monitor blood flow and guide RFA. The application of RFA during this window of reduced tumor blood should increase ablation volume by approximately 27% based on previous mathematical modeling. In the second phase of the present study, a second cohort of athymic nude mice with human HCC xenografts will be treated with combination therapy in this manner. Standard histopathology and immunohistochemistry will be employed to quantify the extent of tumor ablation and the effect of antiangiogenic therapy, respectively.



F. DONG



A. THAKER

TUESDAY
AUDITORIUM

9:00 A.M.

Hypoxia-inducible factor isotypes in physiology and disease**KRISHNA PAREKH**, Duke University School of Medicine

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



K. PAREKH

■ Hypoxia-inducible factors (HIFs) regulate a variety of cellular functions in response to decreases in oxygen tension in the microenvironment. The two isotypes of the HIF α subunit, HIF1 α and HIF2 α , exhibit distinct patterns of expression and target gene specificity. It seems likely that HIF1 is the dominant regulator of physiological vascular development while in the context of malignancy, HIF2 appears to mediate neoplastic growth in certain tumor types such as renal cell carcinoma. In order to determine which plays a predominant role in physiological and pathological conditions, we studied the role of HIF1 and HIF2 in the naturally hypoxic environment of the developing mouse embryo and also in a variety of cell lines, including human kidney, breast and prostate cancer, and endothelial cells.

In order to assess the relative roles of HIFs in embryonic vascular and hematopoietic growth, we pharmacologically inhibited targets uniquely downstream of HIF1 or HIF2 (Notch and TGF- α , respectively) in explanted cultures of the aorta-gonad-mesonephros region from 9.5-dpc wild-type mice. Preliminary data indicate that inhibiting either Notch or TGF- α results in decreased explant area, and furthermore, that Notch inhibition results in decreased vessel length. Hematopoietic progenitor numbers remained constant between both treatment and control groups. Cited2, a transcriptional modulator of HIF1, was hypothesized to promote the predominance of HIF2 in renal cell carcinoma. However, initial experiments demonstrated that Cited2 was not hypoxically upregulated in kidney cancer cell lines. Thus, we have now turned our attention to the role of Cited2 in a variety of other cell types.

HIF1 and HIF2 appear to play significant, yet distinct, roles in embryonic vascular development as identified through inhibition of their unique downstream targets. While Cited2 was not hypoxically regulated in renal cancer cells, we will continue to study its relationship with the HIF isotypes in other tissues.



J.T. ADLER

9:15 A.M.

Histone deacetylase inhibitors upregulate Notch1 and inhibit growth in pheochromocytoma cells**JOEL T. ADLER**, University of Wisconsin School of Medicine and Public Health

Mentor: Herbert Chen, M.D., University of Wisconsin School of Medicine and Public Health

■ Unresectable or malignant pheochromocytoma presents a clinical challenge, as surgery is palliative at best. Thus, new treatments are needed. Activation of the Notch1 signaling pathway has been shown to limit growth and suppress hormonal secretion in neuroendocrine (NE) tumors. Moreover, the histone deacetylase (HDAC) inhibitors valproic acid (VPA) and suberoyl bis-hydroxamic acid (SBHA) have been demonstrated to be strong Notch1 activators. We hypothesized that treatment with these compounds would be an effective strategy to activate the Notch1 pathway and inhibit growth and hormonal secretion in pheochromocytoma cells *in vitro*. To test this hypothesis, pheochromocytoma PC-12 cells were treated with up to 8 mM VPA or 40 μ M SBHA. The NE tumor markers achaete-scute complex-like 1 (ASCL1) and chromogranin A (CgA) were measured by Western analysis. Notch1 activity was measured by Western analysis and a luciferase reporter assay. Growth was assessed by a methylthiazolyldiphenyl-tetrazolium (MTT) bromide cellular proliferation assay, and Western analysis was then used to determine the mechanism of growth regulation.

After 48 hours, treatment with both VPA and SBHA caused a dose-dependent decrease in ASCL1 and CgA, indicating alteration of the NE phenotype and suppression of hormonal secretion. Treatment with both VPA and SBHA led to a threefold induction of active Notch1 protein. After six days, growth was inhibited 70% by both drugs at the highest doses tested ($p < 0.001$). Increased cleavage of caspase-3 and poly-ADP ribose polymerase (PARP) indicated that apoptosis caused the growth inhibition.

This study demonstrates that pharmacologic activation of the Notch1 signaling pathway by the HDAC inhibitors VPA and SBHA is possible in pheochromocytoma cells. Furthermore, *in vitro* treatment of these cells decreased the hormonal markers ASCL1 and CgA and significantly inhibited growth via apoptosis. These drugs may represent a novel therapeutic and palliative strategy in the treatment of patients with unresectable or malignant pheochromocytoma.

9:30 A.M.

Inflammation-induced sonic hedgehog and notch pathways: impact on gliomagenesis**PAULA MCPOLAND**, University of Washington School of Medicine

Mentor: James M. Olson, M.D., Ph.D., University of Washington, and Children's Hospital and Regional Medical Center

■ **Background:** Glioblastoma multiforme (GBM) is the most prevalent brain tumor in the United States and has a median survival of 12 months. Currently, 80–90% of glioma recurrence occurs within two centimeters of the resection margin despite the presence of malignant glial cells throughout the brain at the time of surgery. The sonic hedgehog (SHh) and notch pathways are activated in tissue repair and inflammation and have been linked to the development and maintenance of gliomas.

Hypothesis: 1) GBM recurrence is partly due to postoperative molecular changes, and 2) SHh and notch pathways mediate these changes.

Methods: Stereotaxic partial decortication of C57Bl6 mice was performed. Changes in the SHh and notch pathways were analyzed with real-time PCR, Western blots, and immunohistochemistry. Experiments were repeated in C57Bl6 mice that received a SHh antagonist perioperatively. Finally, we collaborated with Dr. Terry Van Dyke's lab. Two weeks after inducing their transgenic GBM mice, a right-sided partial decortication was performed. The mice were monitored for five months for gliomas.

Results: RNA and protein analysis of surgically manipulated cortex showed no statistically significant differences of the SHh and Notch pathways in comparison to no surgery controls. At four months postinduction, seven control GBM mice showed uniform, bilateral glial hyperproliferation. Two of these had solid tumors, one left-sided and one right-sided. Seven surgically manipulated mice showed higher grade right-sided changes; four of these also showed left-sided glial hyperproliferation. Near the surgical site, one had a grossly visible solid tumor, three showed small foci of solid tumor cells, and three showed increased glial hyperproliferation.

Conclusions: SHh and Notch pathways do not contribute to glioma recurrence postsurgical resection. However, preliminary data suggest that surgical intervention does increase gliomagenesis. It will be essential to investigate other pathways that are both upregulated by inflammation and associated with gliomagenesis.

9:45 A.M.

MicroRNA signatures of differentiating mouse embryonic neural stem cells: implications for glioblastoma multiforme**CHIBA ENE**, Indiana University School of Medicine

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

■ MicroRNAs (miRNAs) are a class of 22-nucleotide noncoding RNAs recognized as important gene regulatory elements in plants and eukaryotes, including humans. Using high-throughput analysis of tumor miRNA, several groups have developed miRNA profiles representing phenotypic signatures of particular cancer types, including breast, colon, pancreatic, and central nervous system neoplasms. Importantly, some profiles are associated with important cellular functions (e.g., differentiation) and disease pathophysiology (e.g., metastatic potential).

Glioblastoma multiforme (GBM) is the most common type of primary malignant brain tumor with an average survival of little more than a year despite aggressive multimodality treatments. Significant improvement in our treatment of patients with GBMs rests on a better understanding of the molecular pathogenesis of these tumors. To that end, our lab and others have recently demonstrated that at least some GBMs arise from a tumor-initiating/tumor stem cell (TSC) that has many of the hallmarks of normal neural stem cells (NSCs). Understanding the differences in genomic and epigenetic regulation of differentiation between TSCs and normal NSCs will be important for understanding the biology of GBMs and ultimately developing better therapies.

Genetically, GBMs tend to be characterized by loss of heterozygosity in 10q (70% cases), EGFR amplification (36%), P16^{INK4a} deletion (31%), and PTEN mutations (25%). Using a mouse model of both normal NSCs and NSCs from genetically engineered mice that harbor some of these major genetic aberrations of GBMs (i.e., INK4A deletion, PTEN deletion), we set out to elucidate signature miRNA profiles associated with specific genomic alterations by performing a comparative analysis of miRNA expression using both normal and genetically engineered mouse NSCs as well as GBM-derived TSCs.

Our results show distinct miRNA signatures associated with different genomic alterations. We believe such profiles will have implications for understanding the role of miRNAs in regulating processes such as differentiation and response to therapy in GBMs.



P. MCPOLAND



C. ENE

TUESDAY
AUDITORIUM

10:00 A.M.

In vitro and in vivo radiosensitization of glioblastoma multiforme by poly (ADP-ribose) polymerase inhibitor, GPI21016**ANDREA L. RUSSO**, Dartmouth Medical School

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



A. L. Russo

■ Glioblastoma multiforme (GBM) has a two-year survival of 3.3%. Radiotherapy, a mainstay of treatment, induces tumor cell killing through double-stranded DNA breaks (DSB). Poly (ADP-ribose) polymerase (PARP) is an enzyme that repairs DSBs. We investigated the in vitro and in vivo effects of the novel PARP inhibitor, GPI21016, on a human GBM cell line (U251).

U251 cells were treated for 6 h with GPI21016 (3 μ M) preirradiation for all in vitro experiments. A chemiluminescent assay evaluated inhibition of PARP-1. Clonogenic survival assays were according to standard protocol. To assess DSBs, γ H2AX foci were assessed at various time points after 2Gy and confirmed by neutral comet assay after 10 Gy. The mechanism of cell death was determined by

immunostaining for mitotic catastrophe (MC) and apoptosis by flow cytometry. Cell cycle changes were evaluated by flow cytometry. For in vivo studies, U251 cells were implanted in subcutaneous and intracranial models.

GPI21016 inhibited PARP activity by 73%. Clonogenic survival resulted in a dose enhancement factor (DEF) of 1.6 at a surviving fraction of 10% (SF=0.49 in drug alone cells) in combination versus IR alone. The combination group versus IR alone had significantly greater γ H2AX foci/cell at 24 h post-IR, DNA damage at 6 h via comet assay, and cells in MC at 72 h post-IR. All treatment groups showed little apoptosis at 24 and 72 h posttreatment. Cell cycle analysis and mitotic index revealed no changes with exposure to drug pre- and post-IR. Median survival in IC mice was greater in the combination versus IR only group (29 days vs. 24 days). In SC mice, there was an additional growth delay of 6 days in the combination (37 days) versus IR group (31 days).

GPI21016 radiosensitizes U251 cells by inhibiting PARP through decreased DSB repair and enhanced MC, and it decreases tumor growth in vivo, suggesting a potential role for this drug in treating GBM.

POSTER C1

Maintenance of bone marrow-derived adult mesenchymal stem cells: the role of interleukin-6

KATIE L. PRICOLA, Stanford University School of Medicine

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

■ Adult human mesenchymal stem cells (MSCs) hold promise for an ever-increasing list of therapeutic uses due to their ease of isolation, expansion, and multilineage differentiation potential. To harness the potential and maximize the clinical relevance of MSCs, the underlying mechanisms by which MSC functionality is controlled must be understood. We have taken a deconstructive approach into understanding the individual components in vitro, namely, the role of candidate “stemness” genes. Specifically, our recent microarray gene expression profiling data have suggested that interleukin-6 (IL-6), a cytokine that is very highly expressed in the bone marrow stroma and well known for its role in bone homeostasis, may be responsible for maintenance of MSCs in their undifferentiated state.

Our results showed that IL-6 gene expression is significantly greater in undifferentiated MSCs as compared to their chondrogenic, osteogenic, and adipogenic counterparts, and that levels of IL-6 are significantly decreased when osteogenic cells are transdifferentiated along the adipogenic lineage. The differential mRNA expression profile has been confirmed by quantitative RT-PCR, with the same trend seen with respect to osteogenic, adipogenic, and chondrogenic differentiation of MSCs. More specifically, we have shown that IL-6 levels are 4-fold and 8-fold greater in MSCs as compared to osteogenic and both chondrogenic and adipogenic cells, respectively. We have also investigated the possible role of IL-6 for the maintenance of MSC “stemness.” Preliminary findings have shown a reproducible trend for MSCs toward both increased proliferation and improved in vitro wound healing upon treatment with exogenous human IL-6, while siRNA-mediated knockdown of IL-6 expression reduces MSC proliferation.

Experiments are under way to explore the influence of IL-6 on differentiation or inhibition thereof, as well as to elucidate the mechanism by which IL-6 exerts these effects on bone marrow-derived MSCs, in particular through the JAK-STAT pathway.

POSTER C2

Cell migration and adhesion in natural three-dimensional extracellular matrices

KIRSI HAKKINEN, Harvard School of Dental Medicine

Preceptor: Kenneth Yamada, M.D., Ph.D., National Institute of Dental and Craniofacial Research, National Institutes of Health

■ Interactions between cells and the extracellular matrix have traditionally been studied in two dimensions in regular tissue culture. However, evidence is accumulating that studying them in three dimensions more closely mimics the in vivo tissue environment. Scientists generally use only one 3D matrix to model how cells adhere in three dimensions. However, no one has systematically compared 3D matrices to see how they differ in their cell-matrix interactions. Four of the most commonly used natural 3D matrices are basement membrane extract (Matrigel), cell-derived matrix, collagen, and fibrin. In this study, the adhesion and migration characteristics of human fibroblasts in these four 3D matrices were examined. We found that fibroblasts migrated fastest in cell-derived matrix and collagen, and migrated slowest in Matrigel. Adhesions made in all four 3D matrices were smaller and more linear than in two dimensions. The integrins and adhesion proteins that fibroblasts use to bind to each matrix are under investigation. This information is valuable for furthering our understanding of development and wound healing and has applications in tissue engineering.

TUESDAY
CLOISTER



K. L. PRICOLA



K. HAKKINEN

POSTER C3

Characterization of novel β -1-adrenergic receptors with preferential β -arrestin-mediated signaling

PRIYESH A. PATEL, Duke University School of Medicine

Mentor: Howard A. Rockman, M.D., Duke University School of Medicine

■ The β -1-adrenergic receptor (β_1 AR) is a G protein-coupled receptor (GPCR) that signals through both classical Gs-mediated and newly identified β -arrestin-mediated pathways. Our lab recently showed that while Gs-mediated signaling promotes harmful cardiomyocyte remodeling in murine models of heart failure, β -arrestin-dependent, Gs-independent signaling confers cardioprotection. Moreover, we showed that β -arrestin-mediated cardioprotection by the β_1 AR involves transactivation of the epidermal growth factor receptor (EGFR). Based on this signaling paradigm, we hypothesize that a novel class of β_1 AR-blockers can be identified that inhibits harmful Gs-mediated signaling while concurrently activating cardioprotective β -arrestin-mediated signaling. In order to further characterize β -arrestin signaling by the β_1 AR, we undertook to alter specific amino acid residues within predicted Gs-interacting domains of the β_1 AR to create novel mutants that signal exclusively through β -arrestin-mediated pathways.

By measuring changes in 1) fluorescent resonance energy transfer (FRET) within a cAMP-reporter protein and 2) β_1 AR-induced cAMP response element binding protein (CREB) activity, we show that our mutant β_1 ARs do not signal through Gs-mediated pathways. Using confocal microscopy and immunoprecipitation experiments, we show that our mutant β_1 ARs recruit β -arrestin in response to adrenergic stimulation. Additionally, we show that our mutant receptors signal through β -arrestin-mediated pathways by measuring agonist-induced extracellular regulated kinase (ERK) phosphorylation as a measure of β -arrestin-dependent transactivation of the EGFR.

Two of our mutant β_1 ARs demonstrate 1) minimal Gs signaling, 2) β -arrestin recruitment, and 3) β -arrestin-dependent pERK activation upon agonist stimulation, thereby confirming that these receptors signal preferentially through β -arrestin-dependent, Gs-independent pathways. These β -arrestin-biased β_1 AR mutants will allow us to better understand β -arrestin-mediated signaling at the β_1 AR and aid in the development of novel β_1 AR-blockers that simultaneously inhibit harmful Gs-mediated signaling while promoting cardioprotective β -arrestin-mediated signaling.



P. A. PATEL



R. GUPTA

POSTER C4

p21^{cip1} inhibits NF- κ B-mediated transcriptional activation of RANTES expression in vascular smooth muscle cells

ROHIT GUPTA, Duke University School of Medicine

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

■ Vascular smooth muscle cell (VSMC) proliferation in vascular proliferative disease results from an imbalance between inflammatory chemokines, such as RANTES, and antiproliferative mediators, such as the cyclin-dependant kinase inhibitor, p21^{cip1}. Our lab has previously shown an increase in VSMC proliferation in *p21*^{-/-} versus wild-type (WT) mice in response to arterial wire injury. It is believed that p21^{cip1} may repress NF- κ B, a transcription factor that promotes RANTES expression. We hypothesized that p21^{cip1} regulates RANTES expression in VSMCs through an NF- κ B-dependant pathway.

Aortic VSMCs from WT and *p21*^{-/-} mice were cultured, and qRT-PCR was performed. RANTES expression was increased 5-fold in *p21*^{-/-} versus WT VSMCs, suggesting a role for p21^{cip1} in regulating RANTES. Cultured *p21*^{-/-} VSMCs were infected with an shRNA construct to knock down p65, an NF- κ B subunit. A 10-fold decrease in RANTES expression in p65 knock-down VSMCs versus control was detected using qRT-PCR. WT and *p21*^{-/-} VSMCs were transfected with a luciferase plasmid containing the RANTES promoter. Luminescence was increased 2-fold ($p < 0.005$) in *p21*^{-/-} VSMCs versus WT. RANTES promoter analysis revealed two probable NF- κ B binding sites. Individual mutation of these sites in the RANTES luciferase plasmid led to a 12-fold decrease in luminescence ($p < 0.001$) in WT versus control VSMCs. A decrease of 27-fold ($p < 0.001$) was observed in *p21*^{-/-} versus control VSMCs, suggesting NF- κ B promotes RANTES transcription, particularly in *p21*^{-/-} VSMCs. Femoral arteries from WT mice were harvested at baseline and 24 hours after wire injury. A >600-fold increase in RANTES expression in injured versus uninjured arteries was detected using qRT-PCR ($p < 0.005$), revealing a potential role for RANTES in the acute postinjury inflammatory response.

RANTES expression in VSMCs is downregulated by p21^{cip1} through a mechanism likely involving NF- κ B. These findings have important implications with respect to the development of novel treatment strategies for neointimal proliferative diseases such as in stent restenosis.

POSTER C5

Quantification of pancreatic islet tissue

VICTOR SANOE HARRISON, University of Hawaii, John A. Burns School of Medicine

Preceptor: David M. Harlan, M.D., National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

■ Diabetes researchers often assume that 1) insulin-producing islets of Langerhans are distributed homogeneously within the pancreas and 2) islets are uniformly affected in disease. To test these assumptions, we modified histological and flow cytometric techniques to study islet cell mass from healthy and diseased mice. We also utilized flow cytometry to sort pancreatic β -cells for studies evaluating β -cell phenotype and gene-expression patterns.

These studies have disclosed several novel observations. First, thorough histological sampling of adult C57BL/6 pancreata reveals increased islet cell mass in the center of the organ compared to the periphery due to increased numbers and size. Second, using two different mouse models of autoimmune diabetes, the nonobese diabetic (NOD) mouse and a transgene-driven model developed in the lab, we notice inhomogeneous islet destruction patterns. Third, we adapted the techniques to characterize islet tumors that arise in multiple endocrine neoplasia type 1 (MEN-1) syndrome by studying various genetically defined mouse MEN-1 disease models.

Our findings demonstrate the need to analyze pancreata thoroughly and understand the limitations of histology and flow cytometry. Efforts to develop in vivo techniques for measuring islet tissue could provide a valuable tool for studying human disease.

POSTER C6

Retinol binding protein 4 regulates pancreatic islet β -cell function

CAMILLE MICHAEL MINDER, Duke University School of Medicine

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

■ Type 2 diabetes is a metabolic disease characterized by peripheral insulin resistance and pancreatic islet β -cell dysfunction. Well-known for its role in transporting vitamin A peripherally from liver stores, retinol binding protein 4 (RBP4) is gaining attention as a mediator of impaired insulin action in skeletal muscle and liver. Serum RBP4 is elevated in insulin-resistant states, including human obesity and diabetes as well as several animal models of diabetes.

To determine whether RBP4 also plays a role in regulating pancreatic islet β -cell function, we modulated RBP4 expression and assessed effects on insulin secretion in pancreatic islets. Three distinct siRNA sequences targeted against RBP4 and delivered via recombinant adenoviruses enhanced glucose-stimulated insulin secretion (GSIS) compared to a virus containing a nonspecific control siRNA sequence in isolated rat pancreatic islets (enhancement of 74%, 108%, and 123%).

Conversely, adenoviral overexpression of RBP4 in islets was associated with impaired GSIS (22–24% decrease). A second set of experiments investigated RBP4 as a physiological regulator of whole-body fuel homeostasis during normal fasting and feeding cycles. During fasting, relative insulin resistance and diminished serum insulin is desirable to avoid hypoglycemia. We tested the effect of a 24-hour fast on RBP4 expression in Sprague-Dawley rats. Fasting for 24 hours was associated with a 2.2-fold increase in serum RBP4 and a 3.7-fold increase in liver RBP4 with unchanged expression in omental adipose tissue compared with rats fed ad libitum.

Taken together, these results suggest that physiologic upregulation of liver and serum RBP4 appropriately signals islets to reduce insulin secretion in the fasted state. However, in obesity, upregulation of RBP4 may contribute to pathophysiology by inducing peripheral insulin resistance and simultaneously interfering with insulin secretion. Our current focus is on overexpression and chemical knockout of RBP4 in vivo in order to assess the physiologic relevance of RBP4 levels on insulin secretion.



V. S. HARRISON



C. M. MINDER

POSTER C7

Role of gangliosides in survival and proliferation of human cutaneous carcinomas

AMRITA ARORA, The University of Chicago Pritzker School of Medicine

Mentor: Amy S. Paller, M.D., Northwestern University, The Feinberg School of Medicine

■ Gangliosides, sialylated membrane glycosphingolipids, have been shown to regulate epidermal cell proliferation and survival. 9-O Acetyl-GD3, an acetylated derivative of GD3, is strongly expressed in disorders of epidermal hyperproliferation such as psoriasis and basal and squamous cell carcinomas, but it is nearly undetectable in the normal epidermis. This finding suggests that 9-O acetyl-GD3 may be implicated in regulating the behavior of skin carcinomas; however, its role in skin disorders is poorly understood. Furthermore, it is unclear whether the observed increase in 9-O acetyl-GD3 in skin disorders is an epiphenomenon or whether this ganglioside promotes increased survival and proliferation. We hypothesized that expression of enzymes that regulate ganglioside content is altered in skin carcinomas, leading to increased 9-O acetyltransferase and decreased GM2/GD2 synthase, and these alterations drive hyperproliferation and increase survival. In order to investigate the effect of changes in 9-O acetyl-GD3 and GM2/GD2 synthase on proliferation and apoptosis of skin carcinomas, keratinocyte-derived SCC12 cells were stably transfected with 9-O acetyltransferase and GM2/GD2 synthase, and studies were performed to assess the effect on cell survival and apoptosis.

Overexpression of 9-O acetyltransferase significantly increased proliferation and survival of the transfected cells, whereas increased GM2/GD2 synthase led to an increased susceptibility to apoptosis. These data suggest that changes in ganglioside expression contribute to increased cell survival and proliferation in skin carcinomas. Thus, increasing GM2/GD2 synthase or blocking 9-O acetylation of GD3 may be potential targets for medical treatment of hyperproliferative skin disorders.



A. ARORA



A. AKHTAR

POSTER C8

Evaluation of possible candidate genes involved in ocular-specific development, including optic fissure closure and retinal pigmentation

AMANA AKHTAR, University of Michigan Medical School

Preceptor: Brian Brooks, M.D., Ph.D., National Eye Institute, National Institutes of Health

■ During the fifth week of normal human gestation, optic fissure closure (OFC) occurs in a manner that is spatially and temporally regulated. Interruption of one or more genes involved in approximation and fusion of the optic fissure edges could lead to closure defect and uveal coloboma—a potentially blinding disease in humans. Gene expression microarray analysis of OFC in mouse yielded a subset of genes thought to be involved in this process. Expression was confirmed through real-time RT-PCR and ISH. Two genes were then selected for investigation: DKK3 (Wnt inhibitor) and FAT4 (giant cadherin). Morpholino (MO) knockdown (k/d) was employed in zebrafish to assay for phenotypes whereby ocular development was affected. FAT4 MO was directed against the ATG initiation site. DKK3 MO disrupted splicing at the Exon4-Intron4 boundary. 1 ng, 2 ng, and 4 ng of each MO were injected in larvae at approximately the one-cell stage and compared to controls.

Ocular phenotype was assessed at 24, 48, and 72 hpf. DKK3 k/d at 1 ng and 2 ng specifically exhibited decreased pigmentation at 24 and 48 hpf as compared to controls, but normalized by 72 hpf. A smaller subset of fish at these concentrations had underventralization and thinning of the lips of the optic fissure, though OFC was unaffected. At 4 ng+, the phenotype was incompatible with life. FAT4 MO injection proved to be lethal at all concentrations.

Both DKK3 and FAT4 are significantly regulated in the mouse at the mRNA level in the correct spatial and temporal pattern to cause OFC defects, though this phenotype was not seen in either k/d. The general lethality of FAT4 k/d indicates its critical involvement in early development and patterning. DKK3 k/d did produce an eye-specific phenotype at low concentrations, and current work has shifted to further define its role in pigmentation.

POSTER C9

The in vitro effects of a single nucleotide polymorphism on expression of extracellular matrix protein Laminin γ -1, a gene important in the pathogenesis of pelvic organ prolapse

VALERIE A. ARBOLEDA, University of California, Los Angeles, David Geffen School of Medicine at UCLA

Mentors: Eric Vilain, M.D., Ph.D., and Larissa V. Rodríguez, M.D., University of California, Los Angeles, David Geffen School of Medicine at UCLA

■ In 2005, our lab identified a C to T single nucleotide polymorphism (SNP) in the promoter region of Laminin γ -1 (LAMC1) in a family with familial pelvic organ prolapse (POP). This SNP lies in the NFIL3/E4BP4 transcription factor binding site of the LAMC1 promoter. This rare mutation was found in 22% of probands with familial POP, while having a frequency of 4.9% in the general population (NCBI dbSNP). In this study, we investigate the role of this rare allele on the in vitro regulation of LAMC1 expression in c2c12 myoblasts, a skeletal muscle cell line.

Preliminary results showed both the C and T allele have a repressive effect on the luciferase expression when compared to the empty promoter vector ($p=0.03$, $p=0.002$). There is a trend toward a moderate difference in the level of repression between the C allele (more often present in controls) and the T allele (more often present in patients with POP). In 45 patients with nonfamilial prolapse, we found the T allele frequency to be 8.9%, almost double the frequency in the general population. Semiquantitative expression analysis of all candidate genes showed differential expression of the laminins between prolapse and nonprolapse controls.

While our preliminary data suggest that this SNP is associated with POP, it is likely that there are many interacting genes that underlie POP pathophysiology. However, our small sample size of patients with nonfamilial POP limits our ability to prove the relevance of our SNP in patients with nonfamilial POP. Using data from large families in conjunction with animal models and functional in vitro studies, we can better understand the role that extracellular matrix proteins, such as laminins, play in this disorder.

POSTER C10

Ciliary dysfunction as an underlying etiology linking primary ciliary dyskinesia with heterotaxy and complex congenital heart disease

MATTHEW SWISHER, Duke University School of Medicine

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

■ Although previously thought to be two distinct pathologies, ineffective mucociliary clearance in the form of primary ciliary dyskinesia (PCD) and complex congenital heart disease (CHD) could have an underlying etiology in ciliary dysfunction. This study arises from our recent finding of an unexpectedly high incidence (40%) of complex congenital heart disease together with heterotaxy in a mutant mouse model of PCD. Heterotaxy is a spectrum of discordant organ situs resulting from aberrant left-right axis. There is increasing evidence that ciliary dysfunction is involved in many different human disorders. Thus, the goal of this study is to elucidate the possible role of ciliary dysfunction in complex CHD associated with heterotaxy.

For this study, we are recruiting subjects undergoing high-risk surgery for complex CHD at Children's National Medical Center. Subjects are being evaluated for PCD using standardized diagnostic tests, including measurement of nasal nitric oxide (NO) production, videomicroscopy of ciliated nasal epithelia, electron microscopy of ciliary ultrastructure, and DNA sequencing for mutations in ciliary proteins known to be associated with PCD or situs anomalies.

To date, we have comprehensively studied five patients with heterotaxy ranging in age from 1 day to 26 years. Cardiac imaging has shown a wide range of cardiovascular anatomies ranging in severity from an interrupted IVC with azygous continuation to an unbalanced AVCD associated with D-TGA and DORV. In addition, abdominal situs has thus far revealed that all patients have splenic abnormalities (either asplenia or polysplenia) in addition to other anomalies such as dextrogastric and midline livers. Ciliary studies have been notable for consistently low NO levels and motion abnormalities ranging from normal to near-complete immotility. Thus, with the recruitment of further subjects and completion of EM and genetic analyses, we hope to implicate ciliary dysfunction in a wide range of complex CHD cases associated with situs abnormalities.



V.A. ARBOLEDA



M. SWISHER

TUESDAY
CLOISTER

POSTER D1

Ovarian inclusion formation in fragile X-associated primary ovarian insufficiency

JOHN JOSEPH DeCARO, Stanford University School of Medicine

Mentors: Stephanie S. Sherman, Ph.D., and Stephen T. Warren, Ph.D., Emory University School of Medicine

■ The fragile X mental retardation 1 (*FMRI*) premutation allele contains 55 to 199 CGG repeats in the 5' untranslated region and is associated with two clinically significant phenotypes: 1) fragile X-associated primary ovarian insufficiency (FXPOI) and 2) fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS, a neurodegenerative disorder predominantly affecting male carriers, is now known to involve an *FMRI* mRNA toxic gain-of-function with hallmark ubiquitin-positive intranuclear inclusions in the brain. The underlying mechanism leading to FXPOI, however, remains unknown. In the current study, we sought evidence for a similar mRNA toxic gain-of-function in FXPOI. Standard pathological techniques and immunocytochemistry were used to examine ovarian tissue from a premutation carrier woman for ubiquitin-positive inclusions. To our knowledge, this is the first such report of ovarian pathology for a premutation carrier.

Ovaries were obtained from a 35-year-old premutation carrier woman in the course of a hysterectomy for a diagnosis of adenomyosis. Hematoxylin and eosin-stained sections demonstrated scant follicles and abundant stroma consistent with primary ovarian insufficiency. Ubiquitin-positive intranuclear inclusion bodies (average measurement 2.2 μm) were observed in stromal cells of both ovaries in a mosaic distribution with the majority of each ovary uninvolved. One ovary was more involved than the other (~ 1.2 inclusions per high power field vs. < 1 inclusion per high power field).

Ubiquitin-positive intranuclear inclusions in ovarian tissue from an *FMRI* premutation carrier similar to those found in FXTAS suggest a common mechanism to both *FMRI* premutation-associated disorders. Further investigation of an *FMRI* mRNA toxic gain-of-function in FXPOI is warranted.



J. J. DeCARO



L. H. PALAVALLI

POSTER D2

Mutational analysis of signal transducing gene families in cutaneous malignant melanoma

LAVANYA H. PALAVALLI, University of Missouri-Columbia School of Medicine

Preceptor: Yarden Samuels, Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Cutaneous metastatic melanoma (MM) is the most common fatal skin cancer, and its incidence has increased at a more rapid rate than any other malignancy in the United States, with more than 60,000 new cases diagnosed each year. Unlike early-stage disease, late-stage melanoma has no curative treatment. The clinical progression of melanoma is assumed to correspond to the accumulation of genetic mutations. In order to develop treatments for advanced disease states, it is important to understand the genetic alterations leading to MM, which will permit personalized design of its treatments.

Our approach for cancer gene discovery involves high-throughput sequence-based mutational analyses and associated bioinformatics that make large-scale searches for somatic mutations feasible. Since our goal is to identify gene mutations in gene families that are potentially amenable to therapeutic intervention, our laboratory is sequencing the tyrosine and serine/threonine kinase pathways (with the collaboration of NISC), the PI3K pathway, and genes that are important for the development and differentiation of melanocytes. Once novel genetic alterations are found, the cellular pathways regulated by these genes will be dissected using genetic, biologic, and biochemical techniques. Since clinical and survival data are available on all patients from whom melanoma samples were originated, correlations between mutational and clinical data will also be performed.

POSTER D3

Effect of C₁-tetrahydrofolate synthetase variants on nutritional and cellular phenotype

KELLY CUSHING, Rush Medical College

Preceptor: Lawrence C. Brody, Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Folic acid plays a critical role in DNA synthesis and nearly all methylation reactions. Perturbations of folate metabolism can produce neural tube defects, congenital heart defects, and increased cancer risk.

A key folate enzyme in the mitochondrion, C₁-tetrahydrofolate synthetase, converts methylene tetrahydrofolate to formyl tetrahydrofolate. The product of this reaction is used in de novo purine synthesis. The gene encoding this enzyme, MTHFD1L, produces two alternatively spliced mRNA transcripts. The shorter splice form creates a nonfunctional protein and is regulated by an intronic (ATT)_n repeat polymorphism.

We hypothesized that genotype of the ATT repeat polymorphism would affect the rate at which metabolites pass through the one carbon metabolic pathway. Given the role of folate in purine synthesis, changes in MTHFD1L activity could influence DNA synthesis and cell division.

Our primary hypotheses related to genetic variants in the C₁-tetrahydrofolate synthetase are 1) alleles of MTHFD1L will influence plasma homocysteine levels (a folate status indicator) and 2) alleles of MTHFD1L will influence purine synthesis and DNA replication. Red blood cell mean corpuscular volume was used as an indirect measure of DNA synthesis.

To answer these questions, we genotyped the ATT repeat polymorphism in 2,523 DNA samples obtained from healthy students attending Trinity College (Dublin). Genotypes were successfully obtained from 2,466 individuals (97.7%).

One-way ANOVA of natural log transformed data yielded the following results when comparing across all six possible genotypes by gender: total homocysteine (males: p=0.95, females: p=0.67), MCV (males: p =0.23, females: p=0.14). Therefore, the repeat polymorphism rs3832406 does not appear to influence plasma homocysteine levels or cellular proliferation. We also observed a previously undescribed allele in six individuals. However, this allele was not associated with any extreme phenotypes.

POSTER D4

Novel mutations in *FLNA* are associated with tetralogy of fallot and aortic aneurysm

NISHANT DINESH PATEL, Johns Hopkins University School of Medicine

Mentor: Harry C. Dietz, M.D., Howard Hughes Medical Institute, Johns Hopkins University School of Medicine

■ *FLNA* mutations have been shown to cause a wide spectrum of developmental anomalies. Loss-of-function mutations in *FLNA*, located on the X chromosome, cause periventricular nodular heterotopia (PVNH) and are embryonic lethal in males, although there are some reports of males with PVNH who have hypomorphic mutations. Filamin A is a cytoskeletal protein that cross-links actin and regulates cell shape and motility. An actin-binding domain is located at the N-terminus. The remainder of Filamin A is composed of 24 repeats that are predicted to adopt β-sheet configurations.

We sequenced *FLNA* in a 7-year-old male (M.M.) with a history of tetralogy of fallot and aortic aneurysm. M.M.'s mother has been diagnosed with PVNH. M.M. was found to have two mutations in the *FLNA* gene. The first was a C to T point mutation found in exon 11 that resulted in an amino acid change from Arg to Cys. The second was a C to T point mutation found in exon 45 that resulted in an amino acid change from Pro to Ser. Forty-eight male and 48 female samples were used as controls, for a total of 144 X chromosomes. M.M.'s mother was also screened and found to have both mutations.

In conclusion, we have found two mutations in the *FLNA* gene in a male patient with tetralogy of fallot, aortic aneurysm, and a family history of PVNH. Recent evidence suggests that Filamin A protein interacts with presenillin (mediates cleavage of Notch receptor and mutations in Notch ligand cause tetralogy of fallot). Evidence also suggests that Filamin A interacts with smad proteins (regulate TGF-β signaling, which is implicated in aortic aneurysm formation). We are currently in the process of screening patients to assess whether *FLNA* is a significant locus for tetralogy of fallot and aortic aneurysm.



K. CUSHING



N. D. PATEL

TUESDAY
CLOISTER

POSTER D5

Action selection in psychogenic movement disorder: an fMRI study

CHRISTINA ANN BREZING, University of Florida College of Medicine

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

■ Psychogenic movement disorder (PMD) is a form of conversion disorder. Behavioral studies in conversion paralysis suggest impairments in motor initiation with prolonged reaction times during a non-motor initiation task. Imaging studies demonstrate hypoactivity of the left dorsolateral prefrontal cortex with attempted movement presumed to be related to initiation impairments and increased self-monitoring with hyperactivity of ventromedial prefrontal cortex and superior temporal cortex during a motor imagery task. Our recent data suggest aberrant motor-limbic interactions during motor initiation with greater pregenual cingulate and dorsal premotor functional connectivity during a psychogenic compared to a voluntary movement (unpublished data). Our goal in this study was to assess brain regions involved in motor action selection in PMD using a functional magnetic resonance imaging (fMRI) task.

In our imaging task, subjects saw a motor planning cue for 2 seconds that indicated either a choice (C: to choose between one of two buttons) or specified (S: to press one of two buttons) movement. Subjects pressed the button during a 1-second red "+," which was followed by a variable fixation jittered for 2 to 4 seconds. 13 PMD and 13 age- and gender-matched healthy volunteers were scanned in a 1.5T fMRI. Standard preprocessing with SPM5 was conducted with head motion parameters used as regressors of no interest in the first-level analysis. The contrasts of C minus S were compared with two sample t tests.

During choice versus specified movement selection, PMD patients had hypoactivity of the right dorsolateral prefrontal cortex, right insula, bilateral precuneus, and right ventromedial prefrontal cortex compared to controls. In contrast to the findings in conversion paralysis, our findings show that during choice motor action selection, PMD patients fail to engage brain regions involved with self-referential processing and with representations of bodily states in addition to the continued observation of hypoactivity of motor initiation regions.



C. A. BREZING



J. M. HALL

POSTER D6

A developmental fMRI study of cognitive flexibility

JULIE M. HALL, University of California, San Francisco, School of Medicine

Preceptor: Ellen Leibenluft, M.D., National Institute of Mental Health, National Institutes of Health

■ One key component of executive function is cognitive flexibility, the ability to adapt to changes in reward contingencies or other environmental factors. Compared to children and adolescents, adults have increased cognitive flexibility and thus are better able to regulate their behavior. To study the development of neural circuitry mediating cognitive flexibility, we used fMRI to compare youths and adults on the neural circuitry engaged during the change task. The change task requires that subjects inhibit a prepotent response and select an alternate one, and has a high error rate. Thus, this task simulates a situation that people encounter frequently and reflects the level of difficulty seen in complex daily tasks.

The sample included 21 healthy adults and 21 healthy youths. We used rapid event-related fMRI and a whole-brain SPM-based analysis with significance set at $p < 0.001$. Results indicate that, compared to youths, adults have increased prefrontal and precuneus activation when successfully completing change trials. Specifically, on successful change versus successful go trials, adults had greater activation in the superior and medial frontal gyri (BA 8) than did youths. Also, on successful change versus unsuccessful change trials, adults activated the precuneus more than youths did. These findings support other studies implicating increased prefrontal activation in the development of cognitive flexibility and further suggest that developmental differences exist in precuneus activation.

POSTER D7

Advanced imaging and intra-arterial thrombolysis in large-vessel strokes

LUIS A. VERDUZCO, Harvard Medical School

Mentor: Ramon G. Gonzalez, M.D., Ph.D., Harvard Medical School

■ Recanalization (RC) may improve outcomes in acute ischemic stroke (AIS), with intra-arterial thrombolysis (IAT) potentially being superior to intravenous tPA in large-vessel occlusions (LVO). Furthermore, MRI diffusion and perfusion (DWI/PWI) mismatch at initial imaging may identify patients who benefit from IAT as it selects for patients with better collaterals. Our aims were to determine whether multitechnique IAT improves RC and outcomes and to evaluate whether RC and time to RC among patients with DWI-PWI mismatch affects infarct growth and outcome.

For both aims, inclusion criteria were 1) IAT within 8 hours of ictus, 2) acute occlusion of the ICA and/or MCA on CT angiogram, 3) follow-up CT or MR imaging, and 4) neurological follow-up. For the second aim, prethrombolysis MR imaging was necessary. Good outcome was defined as modified Rankin score ≥ 2 .

Recanalizers had a good outcome rate of 40% ($p < 0.006$), and all nonrecanalizers had poor outcome. Multitechnique approaches increased RC (78% vs. 48%, $p < 0.02$) but did not alter outcome. Among recanalizers, multitechnique approaches took 50 minutes longer ($p < 0.06$).

Admission DWI volumes above 70 cc led to poor outcome. Among patients below that threshold, a mean time from imaging to RC of 3.3 hours divided the patients into early, late, and non-RC, with corresponding good outcome rates of 78%, 22%, and 0%, respectively ($p < 0.016$), and infarct growth of 18 ± 16 cm³, 67 ± 76 cm³, and 93 ± 90 cm³, respectively ($p < 0.07$).

IAT increases recanalization but may come at the expense of increased time, thus making it necessary but not sufficient for good outcome. Development of pre-IAT clot characterization imaging would inform the interventionalist on what technique to employ for initial thrombolysis attempts as opposed to using different techniques in a linear fashion. In addition, our findings suggest that advanced MR imaging can be used to triage acute stroke patients by identifying those patients who have large initial infarcts due to poor collateral circulation and will do poorly despite recanalization. Furthermore, it identifies patients who will benefit from early recanalization.

POSTER D8

Hypoxic preconditioning reduces cerebral vasospasm and improves functional outcome following experimental subarachnoid hemorrhage

ERIC MILNER, Washington University School of Medicine

Mentors: Gregory Joseph Zipfel, M.D., and David Michael Holtzman, M.D., Washington University School of Medicine

■ Aneurysmal subarachnoid hemorrhage (SAH) results in high rates of morbidity and mortality, a significant portion of which results from secondary neurological injury caused by cerebral vasospasm. Aside from the modest benefit of nimodipine, no medical therapy has thus far proven efficacious against this serious SAH-induced complication. Cerebral preconditioning refers to the concept whereby the brain's inherent resistance to ischemic injury can be augmented by prior exposure to a sublethal preconditioning stimulus. Though most investigations have focused on neurons as the target of cerebral preconditioning, several recent reports suggest that the cerebrovasculature itself may also be a key component to the observed neuroprotection. We thus sought to evaluate the effect of hypoxic preconditioning (HP) on cerebral vasospasm as well as neurological deficits following experimental SAH. Adult male C57BL/6 mice underwent HP by exposure to 8% oxygen (balance nitrogen) for 4 hours. SAH was induced 24 hours later via endovascular perforation. Functional outcome was assessed daily utilizing rotarod latency test and sensorimotor score. Cerebral vasospasm was assessed 72 hours post-SAH via middle cerebral artery (MCA) diameter measurements obtained following pressure-controlled casting of the cerebrovasculature. Weight, neurological outcome, and cerebral vasospasm were compared by repeated measures ANOVA. Values of $p < 0.05$ were considered statistically significant.

Exposure to HP prior to experimental SAH led to near-complete prevention of cerebral vasospasm (average MCA diameter ipsilateral to endovascular perforation was $70 \pm \mu\text{m}$ in non-HP animals vs. $102 \pm 4 \mu\text{m}$ in HP animals; average MCA diameter in unoperated animals was $104 \pm 5 \mu\text{m}$). Moreover, HP significantly improved rotarod performance and neuroscore following experimental SAH.

These results suggest that augmentation of inherent neurovascular resistance via preconditioning stimuli may be a new strategy toward prevention and/or reduction of SAH-induced cerebral vasospasm. Future studies are needed to investigate clinically relevant preconditioning strategies as well as underlying molecular mechanisms.



L. A. VERDUZCO



E. MILNER

POSTER D9

Netrin-4 enhances angiogenesis and neurological outcome after cerebral ischemia

STANLEY HOANG, Stanford University School of Medicine

Mentor: Gary K. Steinberg, M.D., Ph.D., Stanford University School of Medicine

■ Functional recovery following cerebral ischemia is governed by plastic processes that result from the induction of axonal outgrowth, the restoration of synaptic architecture, and the regeneration of vascular networks. Netrin-4 has been implicated as both a synaptogenic and angiogenic factor that promotes neurite outgrowth and angiogenesis. This dual function of netrin-4 suggests that it may be important for promoting functional recovery following cerebral ischemic injury. In this study, we investigated the expression of netrin-4 and its putative receptors, DCC and Unc5H1, following distal middle cerebral artery occlusion (dMCAO) in mice. Netrin-4 recombinant protein was also administered via an osmotic minipump intracerebroventricularly to examine its effect on angiogenesis and behavioral recovery.

Netrin-4 protein was highly upregulated in the ischemic core as soon as one day after cerebral ischemia, with subsequent downregulation after one week. Its expression was limited to the area of blood-brain barrier damage, as demonstrated by the presence of Evans blue. Netrin-4 protein expression was seen in both blood vessels and astrocytic foot processes, which suggests an important role of netrin-4 in blood-brain barrier repair and angiogenesis. While there was no significant upregulation of the putative netrin-4 receptor Unc5H1, there was a significant increase in the expression of DCC in the ischemic penumbra. DCC protein was also found to be localized to neuronal processes, which may suggest a role in neurite sprouting. Importantly, intracerebroventricular administration of netrin-4 into the ischemic brain increased blood vessel density and endothelial cell proliferation and improved behavioral outcome at different time points after stroke.

These findings suggest that netrin-4 may improve poststroke functional recovery by enhancing blood vessel proliferation and angiogenesis. It may also have an effect on neurite sprouting and synaptogenesis through its interaction with the DCC receptor. These properties make netrin-4 an ideal candidate to improve behavioral recovery after ischemic injury.

POSTER D10

The role of inflammatory cytokines in altered fetal neural progenitor cell behavior

ANDRA L. DINGMAN, Stanford University School of Medicine

Mentor: Theo D. Palmer, Ph.D., Stanford University School of Medicine

■ Infection and inflammation during pregnancy are correlated with adverse neurological outcomes in the child. The goal of this study is to investigate the effect of maternal inflammation on fetal neural progenitor cell (NPC) proliferation and differentiation and to determine if specific cytokines are responsible for alterations in fetal neurogenesis. Pregnant mice were injected with lipopolysaccharide (LPS) on embryonic day (E) 12 or 14, and cells in S-phase were labeled two hours later with 5-bromo-2-deoxyuridine (BrdU). At E12, maternal inflammation causes a high rate of fetal death at low doses of LPS (60 $\mu\text{g}/\text{kg}$), but surviving fetuses show normal BrdU incorporation in the brain. In contrast, LPS treatment at E14 is relatively well tolerated at doses up to 200 $\mu\text{g}/\text{kg}$ LPS, but the fraction of BrdU-labeled cells in fetal brains is reduced. To determine the direct effect of cytokines, we cultured mouse NPCs with cytokines and examined the fraction of S-phase cells. Treating cells with transforming growth factor-beta (TGF- β) reduces the mitotic index, and tumor necrosis factor-alpha (TNF- α) reduces neuronal differentiation. Our in vivo data suggest that maternal inflammation at E14 reduces fetal neurogenesis, and our in vitro data suggest that these effects may be due to the direct actions of cytokines. We are currently investigating the cytokine profile in maternal serum, placenta, and fetal brain to see if these cytokines are increased, and whether treatment of dams with TGF- β and/or TNF- α is sufficient to reproduce alterations in fetal neurogenesis.



S. HOANG



A. L. DINGMAN

POSTER D11

In vivo manipulation of Kv4.2 expression and subsequent electrophysiologic characterization in hippocampal CA1 pyramidal cells

ABIGAIL RAO, Dartmouth Medical School

Preceptor: Dax Hoffman, Ph.D., National Institute of Child Health and Development, National Institutes of Health

■ Pyramidal cells, which are the major excitatory projection neurons in regions such as the hippocampus, receive and process thousands of synaptic inputs at the spines of their dendrites. The integration of these inputs, and how experience-dependent changes at synapses alter such integration, is not well understood. Whereas much research on synaptic plasticity has focused on glutamate receptors, the presence of voltage-gated ion channels in the dendrites of hippocampal CA1 pyramidal cells suggests that voltage-gated ion channels also underlie synaptic plasticity. Our work has focused on Kv4.2, a voltage-gated potassium channel subunit that is highly expressed in CA1 dendrites and is the molecular identity of the rapidly inactivating (A-type) potassium current. This current has been shown to shape both incoming synaptic signals and back-propagation of action potentials in CA1 dendrites. Previous work in the lab has shown that, in organotypic slice cultures, the expression level of Kv4.2 affects the levels of long-term potentiation that can be achieved, particularly via effects on NMDA receptor (NMDAR) subunit composition. Given that the subunit composition and channel properties of the NMDAR change during postnatal development, we sought to explore whether expression levels of Kv4.2 affect NMDAR subunit composition at different postnatal ages. Expression levels of Kv4.2 in CA1 pyramidal cells were altered via stereotaxic injection of a modified Sindbis virus into the brains of wild-type mice. This virus has been engineered by our lab to encode fluorescently tagged versions of Kv4.2. Following stereotaxic injection of mice, acute slices were prepared from the brains of these animals, and electrophysiology was performed to assess A-current density and NMDA-evoked currents. Preliminary evidence suggests a relationship between postnatal age, Kv4.2 activity, and NMDAR activity, implicating Kv4.2 in activity-dependent changes in synaptic plasticity.

POSTER D12

Melanopsin bistability and its implications on intrinsically photosensitive retinal ganglion cell firing in circadian rhythm entrainment

KAREEM MAWAD, Washington University School of Medicine

Mentor: Russell N. Van Gelder, M.D., Ph.D., University of Washington School of Medicine

■ In mammals, intrinsically photosensitive retinal ganglion cells (ipRGCs) transmit nonvisual phototic information to the suprachiasmatic nuclei, resulting in photoentrainment of the central circadian clock. These cells employ melanopsin, a retinaldehyde-based opsin protein, as a photopigment. Unlike other vertebrate opsins, the retinal pigment epithelium-based enzymatic photocycle for chromophore regeneration is not necessary for continued melanopsin-based signaling. Comparative sequence homology indicates that melanopsin is more closely related to invertebrate photopigments than to vertebrate rhodopsin and cone opsin. Heterologous expression studies of melanopsin provide evidence that, like some invertebrate photopigments, the melanopsin chromophore is able to isomerize from *cis*- to *trans*-isomer and *trans*- to *cis*-isomer by sequential photon absorption. A bistable pigment regenerating functional photopigment through intrinsic photoisomerase activity could account for the independence of melanopsin from the enzymatic photocycle. A bistable pigment would be expected to show sensitization following pretreatment with appropriate wavelength light, and a nonunivariant action spectrum. We have tested this hypothesis by performing multi-electrode array recordings of murine retina using sequential wavelength light stimulation.

ipRGC firing in response to equivalent photon flux of equipotent wavelengths demonstrates identical signaling consistent with an opsin having a single absorption peak. Responses to two test pulses flanking an intervening pulse of potentially regenerative-wavelength illumination show statistically equivalent responses, again arguing against second photon absorption and photoregeneration of pigment. Retinaldehyde depletion and repletion studies suggest that ipRGCs are capable of functional chromophore regeneration with minute concentrations of retinaldehyde.

Our results suggest that murine ipRGC responses reflect a univariantly responsive photoreceptor that does not undergo photoregeneration *ex vivo*. *In vivo* studies have suggested that long-wavelength light may potentiate subsequent short-wavelength responses. We do not find evidence that this potentiation is due to photoreversal of melanopsin. Uptake of local chromophore may provide the mechanism for inner retinal pigment regeneration.



A. RAO



K. MAWAD

WEDNESDAY
ROOM A

9:00 A.M.

Myeloperoxidase-catalyzed carbamylation of low-density lipoprotein and its interaction with scavenger receptor class A type I: a physiologically relevant mechanism promoting cardiovascular disease**ROBERT A. KOETH**, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentor: Stanley Hazen, M.D., Ph.D., Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

■ Smoking is a well-documented risk factor for cardiovascular disease. Similarly, a member of the hemeperoxidase superfamily, myeloperoxidase (MPO), is associated with multiple proatherogenic pathways, including creation of oxidized low-density lipoprotein (LDL) with cigarette smoke extract. Recently, we have identified a novel chemistry involving MPO and LDL. MPO catalyzes a reaction between cosubstrate thiocyanate, a compound elevated in smokers, and hydrogen peroxide to cyanate, a molecule capable of modifying LDL by carbamylation. Interestingly, carbamylated LDL has been shown to bind to scavenger receptor class A type 1 (SR-A1) and induce foam cell formation. Moreover, plasma levels of homocitrulline (carbamylated lysine) were shown to predict major adverse cardiac events. Here, we test the hypothesis that carbamylated LDL is an endogenous ligand for SR-A1 *in vivo*. Furthermore, we test the hypothesis that interaction of carbamylated LDL (cLDL) with SR-A1 promotes atherogenic macrophage pathology.

Our studies demonstrate that progressive carbamylation at physiological levels is able to increase SR-A1 recognition of LDL. In ongoing studies, we are investigating the interaction of SR-A1 with cLDL and its ability to induce cholesterol accumulation and apoptosis in macrophages. Furthermore, through proteomics studies we hope to identify site-specific modification(s) involved in SR-A1 recognition of cLDL.

These results suggest cLDL is a potential mediator of macrophage cell dysfunction and atherosclerotic disease progression. Moreover, they provide further evidence of the link between inflammation and cardiovascular disease.



R. A. KOETH



M. PATEL

9:15 A.M.

The role of endoplasmic reticulum stress in age-related macular degeneration**MRINALI PATEL**, Duke University School of Medicine

Preceptor: Chi-Chao Chan, M.D., National Eye Institute, National Institutes of Health

■ Glycoprotein accumulation in age-related macular degeneration (AMD)-associated drusen deposits, alterations of chaperone proteins in human AMD and in the *Ccl2^{-/-}/Cx3cr1^{-/-}* double knockout (DKO) murine model of AMD, protein misfolding in AMD-related inherited maculopathies, and endoplasmic reticulum (ER) stress induction by oxidative stress and hypoxia—two key pathological processes in AMD—suggest a role for ER stress in AMD. This study sought to determine the role of ER stress in the DKO model of AMD and to assess the ability of celastrol, a triterpene that upregulates heat shock proteins, to induce a cytoprotective effect in the retinas of DKO mice. ER stress levels were determined in DKO and age-matched wild-type (WT) mice by immunohistochemistry for ER stress markers p-PERK, p-eIF2 α , CHOP, and p-IRE1 α and by quantitative PCR for *CHOP* mRNA. In addition, DKO and WT mice were treated by intraperitoneal injection for four weeks with celastrol or vehicle. Fundoscopy was conducted prior to and after treatment, and the eyes from the mice were harvested for histology, ultrastructural analysis, immunohistochemistry, and microdissection of the retinas for mRNA extraction. *In vitro* studies were performed to assess the effect of celastrol treatment on hsp40, hsp70, and hsp90 protein expression. Compared to WT, DKO mice exhibit diffusely increased p-PERK, p-eIF2 α , p-IRE1 α , and CHOP protein expression in the retina and increased *CHOP* mRNA expression. *In vitro*, celastrol treatment upregulated the expression of heat shock proteins. Treatment of DKO mice with celastrol, as compared to vehicle, reduced protein expression of ER stress markers p-PERK, CHOP, and p-IRE1 α and withheld clinical progression of AMD lesions, as determined by number and size of subretinal and deep retinal lesions. These findings demonstrate that the *Ccl2^{-/-}/Cx3cr1^{-/-}* murine model of AMD exhibits increased ER stress and responds to cytoprotective strategies such as celastrol, implicating a role for cellular stress reduction in AMD.

9:30 A.M.

Small-molecule inhibition of Hedgehog signaling in adult mouse bone homeostasis**JOSHUA A. GORDON**, University of California, Los Angeles, David Geffen School of Medicine at UCLA

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

■ Hedgehog signaling is known to be required for osteoblast differentiation in endochondral bone formation. Recent studies have demonstrated a novel role for the pathway in mature osteoblasts during regulation of adult bone homeostasis. Osteoblast-specific knockout mice lacking the gene encoding Smoothed (Smo), a transmembrane protein required to transduce hedgehog signaling, have decreased bone formation and resorption. In later life these mice demonstrate a reduction in bone loss. Conversely, osteoblast-specific mutant mice lacking the gene encoding Patched, a transmembrane protein that catalytically inactivates smoothed, show increased bone formation and resorption, which results in low-quality and fragile bone. Cell and molecular studies have demonstrated that PTHrP expression is upregulated by Hedgehog signaling. Here, we utilize cyclopamine and statins, known inhibitors of the Hedgehog signaling pathway, to see if small-molecule inhibition of the pathway can alter bone homeostasis, substantiating a novel drug target for bone-related disease.

Aged wild-type (WT) C57/Black6 mice were fed with simvastatin-containing food at a dose of 50 mg/kg and were treated for a period of three months. Another group of mice treated with simvastatin were treated for three months and received a higher dose regiment for an additional month. Mice treated with cyclopamine received injections at a dose of 1.25 mg/kg three days a week for one month. Harvested mice will be analyzed using micro-CT, DEXA scans, bone histology, qPCR of hedgehog downstream genes and ELISA assays for P1NP and CTX bone turnover markers.

Preliminary data from dEXA bone analysis suggest that mice treated with low-dose simvastatin demonstrate similar bone mineral density as compared with age-matched control mice. Ongoing analysis will continue to elucidate hedgehog function in adult bone homeostasis.

9:45 A.M.

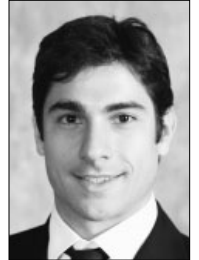
BMP-mediated bone regeneration and its interactions with the Wnt pathway**STEVE MINEAR**, Stanford University School of Medicine

Mentor: Jill Helms, D.D.S., Ph.D., Stanford University School of Medicine

■ Bone regenerates throughout life, during remodeling and during repair. To gain insights into this regeneration, we implemented a stereotypical model of repair and evaluated the contribution of bone morphogenetic protein (BMP) signaling to the healing process. We recently found that Wnt3a accelerates intramembranous ossification in this model; here, we examined how BMP affects skeletal regeneration. We created trans-cortical tibial injuries and treated them with either BMP2 or PBS. In PBS-treated tibiae, skeletal progenitor cells in the periosteum undergo a limited amount of chondrogenic differentiation while progenitor cells within the bone marrow cavity differentiate into osteoblasts to form the bony regenerate. In BMP-treated tibiae, progenitor cells in the bone marrow cavity did not differentiate into osteoblasts. Even after two weeks, BMP-treated bone marrow cavities were devoid of osteoblasts. Furthermore, in BMP-treated tibiae, progenitor cells from the periosteum and adjacent muscle differentiated into chondrocytes and formed an enormous (31-fold larger) chondrogenic domain.

We found clues as to how BMP treatment affected bone marrow progenitor cells: on postsurgical day 3, BMP-treated tibiae also showed reduced Wnt responsiveness in the marrow cavity, and on day 6, BMP-treated tibiae showed reduced Wnt2b and Wnt3a expression relative to PBS controls. These data suggest that BMP treatment represses a Wnt-mediated osteogenic stimulus.

Taken together, our data suggest that there are two populations of skeletal progenitor cells in an injury environment and that these populations respond to BMPs in distinctly different ways. Periosteal progenitors react to BMP by differentiating into chondrocytes and heal the defect via endochondral ossification. In contrast, BMP appears to arrest osteoblast differentiation of bone marrow progenitors, and we propose a model whereby this occurs via repression of Wnt signaling.



J. A. GORDON



S. MINEAR

WEDNESDAY
ROOM A

10:00 A.M.

Purification and systemic injection of murine bone marrow stromal cells**BRIAN SWORDER**, Boston University School of Medicine

Preceptor: Pamela G. Robey, Ph.D., National Institute of Dental and Craniofacial Research, National Institutes of Health

■ Bone marrow stromal cells (BMSCs) consist of the non-blood-forming cells of the bone marrow and contain a subset of postnatal stem cells, termed skeletal stem cells (SSCs). Multiple studies have shown that these cells have the potential to differentiate into bone, hematopoietic supportive stroma, adipocytes, and cartilage. BMSCs are traditionally isolated from other cells in the marrow by their ability to adhere to tissue culture plastic. However, when this strategy is employed with murine BMSCs, a significant number of macrophages also adhere, thereby giving rise to a mixed population of BMSCs and macrophages.

In order to allow for further experimentation using murine BMSCs, magnetic cell separation was used to isolate the CD45/CD11b-negative cells, thereby eliminating the macrophage portion of these cultures. Once this fractionated BMSC population was obtained, the cells were characterized to determine if their growth and differentiation potential were retained following macrophage elimination. Fractionated and unfractionated murine BMSC cultures were exposed to osteogenic and adipogenic differentiation media *in vitro*, and their respective differentiation potentials were compared. Further, fractionated and unfractionated murine BMSCs, with a hydroxyapatite/tricalcium phosphate scaffold, were transplanted subcutaneously into immunocompromised mice in order to assay their *in vivo* differentiation potential.

Finally, bone marrow stromal cells and more specifically, skeletal stem cells, have been postulated to have therapeutic potential for diseases of the skeletal system and beyond. In order to investigate this potential, we ovariectomized wild-type mice to create a state of high bone turnover. Following this, purified murine BMSCs labeled with green fluorescent protein (GFP) were injected systemically. Two weeks following systemic injection, these mice were sacrificed. Their long bones were analyzed by micro-CT to assay for changes in bone density and were also analyzed by immunohistochemistry in order to determine if the systemically injected cells had engrafted in the bone marrow.



B. SWORDER



A. K. DURRANI

10:15 A.M.

Stromal cell-derived factor 1 α (SDF-1 α) expression by intravenously administered mesenchymal stem cells to rats after stroke injury: effect on neuroprotection, neurological outcome, and angiogenesis**AMIR KHAN DURRANI**, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentor: Marc S. Penn, M.D., Ph.D., Bakken Heart-Brain Institute, Cleveland Clinic

■ Stromal cell-derived factor 1 α (SDF-1 α) expression is increased in the penumbra of the ischemic brain and is involved in hematopoietic stem cell migration from bone marrow to peripheral blood. SDF-1 also demonstrates growth and survival benefits in CXCR4-expressing mesenchymal stem cells (MSC). Recently, it was shown that intracerebral administration of SDF-1 α resulted in neuroprotection from neurotoxic insult, and induced increased bone marrow (BM)-derived cell migration to the ischemic brain, reducing the volume of cerebral infarction and improving neural plasticity. MSC constitutively express SDF-1 α ; therefore, in an attempt to define the trophic effects of intravenously administered MSC through SDF-1 α , we generated MSC that overexpressed SDF-1 α . We then compared the effects of saline-, MSC-, and SDF-1 α -overexpressing MSC on neuronal and glial cell survival and regeneration, angiogenesis, neurological outcome, and MSC survival. To measure effect of MSC administered *i.v.* on rats subjected to permanent middle cerebral artery stroke and temporary bilateral carotid occlusion, we injected saline-, GFP-labeled MSC-, and GFP-labeled SDF-1-overexpressing MSC into the tail vein 30 minutes after carotid reperfusion and sacrificed rats 7 days later. The Montoya test was used to evaluate neurological function, and 2,3,5-triphenyltetrazolium chloride staining was used to detect and quantify cerebral infarction. The distribution of injected cells in brain was analyzed in recipient rats using immunohistochemical staining. To determine the fate of MSC, immunofluorescence double labeling for GFP and neuronal and glial markers was done.

9:00 A.M.

Alcohol consumption and risk of breast cancer in postmenopausal women: the NIH-AARP diet and health study

QUAN LAN JASMINE LEW, The University of Chicago Pritzker School of Medicine

Preceptors: Arthur Schatzkin, M.D., Dr.P.H., and Yikyung Park, Sc.D., National Cancer Institute, National Institutes of Health

■ Although epidemiologic studies have consistently shown an association between alcohol use and invasive postmenopausal breast cancer, observational studies of this association according to hormone receptor status have been sparse.

We analyzed 184,418 postmenopausal women (mean baseline age of 62 years) in the prospective NIH-AARP Diet and Health Study. At baseline, we assessed alcohol and other nutrient intakes with a food frequency questionnaire, and collected data on demographics, lifestyle, and medical history. We used Cox proportional hazards models to calculate relative risks (RR) and 95% confidence intervals (CI), adjusting for age, race, height, body mass index (BMI), physical activity, smoking history, parity, age at first birth, age at menopause, family history of breast cancer, frequency of breast biopsy, hormone replacement therapy (HRT) use, and intakes of fat, folate, and energy.

During an average of seven years of follow-up, we identified 5,461 invasive breast cancer cases. 30.0% of women did not drink alcohol; the mean alcohol intake among drinkers was 8.2 g/day (10th–90th percentile=0.4–20.1 g/day). Alcohol was associated with an increased risk of breast cancer: compared to no alcohol, the multivariate RR (95% CI) for ≥ 35 g/day of intake was 1.35 (1.17–1.91) ($p < 0.001$). ER+/PR+ tumor types showed a stronger association with alcohol than ER-/PR- tumor types: compared to no alcohol, the multivariate RR (95% CI) for ≥ 35 g/day was 1.46 (1.12–1.91) for ER+/PR+ tumors ($n=1,641$), 0.80 (0.40–1.62) for ER-/PR- tumors ($n=366$), and 1.59 (0.90–2.78) for ER+/PR- tumors ($n=336$). No significant alcohol interactions were observed with BMI, HRT use, family history of breast cancer, and folate intake in relation to breast cancer risk.

Alcohol consumption, even at moderate levels, increased the risk of breast cancer. This positive association was largely confined to ER+/PR+ tumors, suggesting that hormonal factors are mediating the positive alcohol-breast cancer association.

9:15 A.M.

Determinants of seroconversion among HPV-16- and HPV-18-infected women in the enrollment phase of the HPV-16 and -18 vaccine trial in Guanacaste, Costa Rica

CHRISTINA BENNETT, Indiana University School of Medicine

Preceptor: Allan Hildesheim, Ph.D., National Cancer Institute, National Institutes of Health

■ HPV, a very common sexually transmitted infection, is the causative agent for the development of cervical cancers. One biomarker of HPV infection is seropositivity. However, not all women infected with HPV seroconvert.

The objective of this analysis was to investigate the determinants of seroconversion among HPV-16- and HPV-18-infected young women in a community-based trial of an HPV-16 and -18 vaccine in Guanacaste, Costa Rica.

Included in this evaluation were 646 sexually active women known to be HPV-16 and/or HPV-18 infected at enrollment into the trial (prevaccination). These women were given medical and pelvic examinations and were also asked to complete a medical questionnaire. Cervical swabs were used for HPV DNA detection by an SPF₁₀ DNA enzyme immunoassay, and serum was tested for anti-HPV L1 antibodies by enzyme-linked immunosorbent assay (ELISA), for both HPV-16 and -18.

To identify determinants of HPV seropositivity among infected individuals, we assessed the association between demographic, social, and behavioral risk factors with HPV-16 and HPV-18 seropositivity by estimating odds ratios and their respective 95% confidence intervals using logistic regression models.

Of all demographic and behavioral risk factors measured in multivariate analyses, number of partners (recent and total), number of pregnancies (two or more pregnancies), contraception use (ever use pill and/or injectables; duration of pill use), number of months since sexual debut, and total number of months with the most recent partner were all statistically significantly associated with HPV-16 seropositivity. A modest association was observed between smoking (current) and seropositivity. We also observed that increasing DNA viral load and cytological abnormalities were associated with an increased likelihood of HPV-16 seroconversion. More modest effects were observed for HPV-18.



Q. L. J. LEW



C. BENNETT

WEDNESDAY
ROOM B

9:30 A.M.

Elucidating the role of variants in insulin-like growth factor 2 mRNA binding protein 2 in the pathogenesis of type 2 diabetes

JESSE J. HANISCH, Creighton University School of Medicine

Mentor: Francis S. Collins, M.D., Ph.D., National Human Genome Research Institute, National Institutes of Health

■ Variants in the insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*) gene are associated with the risk of developing type 2 diabetes (T2D) in genome-wide association studies conducted by the Finland-United States Investigation of NIDDM Genetics (FUSION), Diabetes Genetics Initiative (DGI), and Wellcome Trust Case Control Consortium/UK Type 2 Diabetes Genetics Consortium (WTCCC/UKT2D) research groups. *IGF2BP2* contains six RNA-binding domains and belongs to a family of RNA binding proteins that recognize and bind specific “zip-code” sequences within target mRNAs. By binding to unprocessed mRNAs, *IGF2BP2* and other proteins in its family can tightly regulate mRNA stability, transport, localization, and utilization. To investigate the role of *IGF2BP2* in the development of T2D, various genetic and functional studies were performed. Five prime rapid amplification of cDNA ends (5' RACE) analysis detected several novel splicing isoforms of this RNA-binding protein. An isoform encoding five RNA-binding domains appears to be more abundant in the presence of the T2D risk allele. Using RNA immunoprecipitation combined with microarray analysis of transcripts (RIP-Chip), we identified the target RNAs of endogenous *IGF2BP2* in human fetal kidney 293T cells. In addition, a recombinant version of this approach (rRIP-Chip) with particular splicing isoforms of *IGF2BP2* cloned into an expression vector was used to capture the target mRNAs of the various *IGF2BP2* splicing forms. Several target mRNAs identified by both forms of the RIP-Chip assay were found to be highly expressed in pancreatic islets and involved in various molecular pathways related to diabetes. Further studies of *IGF2BP2* and its target mRNAs will be required to further elucidate the role of *IGF2BP2* in the pathogenesis of T2D.



J. J. HANISCH



R. WIRKA

9:45 A.M.

Mechanisms of atrial fibrillation: common polymorphisms in the connexin-40 gene *GJA5*

ROBERT WIRKA, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Mentors: Jonathan D. Smith, Ph.D., and David Van Wagoner, Ph.D., Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

■ Atrial fibrillation is characterized by multiple small electrical reentrant circuits in the atria that cause the atria to contract in a rapid, uncoordinated fashion. Slowed, non-uniform atrial conduction, which favors the initiation and perpetuation of these reentrant circuits, can be caused by a number of pathological conditions, including myocardial infarction. However, defects in connexin-40 (*cxn40*), an atrial-specific gap junction protein with profound effects on inter-myocyte conduction, have also been implicated in abnormalities of conduction. A common promoter polymorphism in the *cxn40* gene *GJA5* has been associated with AF in case-control studies, and has been suggested to decrease *GJA5* promoter activity, possibly leading to impaired expression of *cxn40*. We sought to determine the effect of this promoter polymorphism on the quantity of *GJA5* transcript produced in human atrial tissue.

Allelic expression imbalance (AEI), a technique that allows the separate but simultaneous quantification of each allelic mRNA in a heterozygous sample, was used to compare human atrial mRNA levels produced by the major and minor promoter alleles of *GJA5*. Total RNA and genomic DNA (gDNA) were isolated from eight human atrium samples. Allelic discrimination and quantification of cDNA and gDNA was achieved by real-time PCR using allele-specific fluorescent probes (TaqMan method).

The results of my experiments to date suggest that the *GJA5* promoter polymorphism does not result in allelic expression imbalance in heterozygous individuals. However, we discovered a novel insertion polymorphism in the *GJA5* 3' untranslated region (UTR) with an allele frequency of ~0.46 that does result in allelic imbalance. Thus, our data to date suggest that this 3'UTR length polymorphism has an effect on transcription and/or mRNA stability in vivo. We are currently performing AEI analysis on additional atrium samples, and we are conducting a case-control study to test for an association of this polymorphism with AF.

10:00 A.M.

Genetic susceptibility to murine therapy-related acute myeloid leukemia**RYAN KEVIN FUNK**, Washington University School of Medicine

Mentor: Timothy A. Graubert, M.D., Washington University School of Medicine

■ Therapy-related acute myeloid leukemia (tAML) arises 3–5 years posttherapy in 1–10% of patients exposed to alkylator chemotherapy and has only a 6- to 12-month median survival. Prior studies suggest that tAML susceptibility has a genetic component, but specific variants that influence susceptibility are poorly understood. Our lab previously identified mouse strains that are susceptible or resistant to developing tAML. To map the genetic basis of tAML susceptibility, we designed an F₂ intercross experiment between resistant (C57BL/6J × C3H/HeJ) and susceptible (SWR/J) strains. To increase the sensitivity of mapping experiments, all mice carry one copy of the leukemia-initiating hCG-PML-RARA transgene. F₂ mice were treated (n=141) or not treated (n=141) with the potent alkylator ENU and sacrificed and analyzed when moribund. We also analyzed treated (n=21) and untreated (n=24) mice from a resistant (C57BL/6J × C3H/HeJ) background for comparison to F₂ mice. To facilitate quantitative trait locus (QTL) mapping, we genotyped tail DNA from F₂ mice at 384 informative SNPs evenly spaced across the genome. We performed QTL analysis using time-to-leukemia, spleen weight, and WBC as quantitative traits.

Untreated F₂ mice developed leukemia earlier (108 days vs. 234 days) and more frequently (79.4% vs. 12.5%) than mice from the resistant background. ENU treatment of F₂ mice increased the incidence (90.4%) and decreased median survival (151 days vs. 254 days) compared to untreated F₂ mice. Significant QTLs (five survival, five spleen weight, and three WBC) were found on eight chromosomes. Candidate QTL genes from these intervals include *Sfp1*, *Nf1*, *Trp53*, and *Bcl2*.

Our findings confirm that SWR/J mice carry increased tAML susceptibility alleles. Multiple QTLs for each phenotype suggest that tAML susceptibility is complex and governed by genes at several loci. Identification of specific variants influencing tAML susceptibility will require further studies. Understanding tAML susceptibility could help reduce the incidence of this devastating disease.

10:15 A.M.

An effort to create a factor IX expression vector that allows sustained gene expression through the placement of nucleosome positioning signals**LIA EUNHEE GRACEY**, University of Michigan Medical School

Mentors: Andrew Z. Fire, Ph.D., and Mark A. Kay, M.D., Ph.D., Stanford University School of Medicine

■ One of the major problems with nonviral gene transfer is that foreign transgenes are efficiently silenced after initial expression in mammalian systems. Many questions have been asked about the role of DNA sequence and the molecules involved in chromatin modulation, but there is little knowledge of what exactly controls where nucleosomes sit on DNA and how this knowledge can be applied to therapeutic vector design.

We are currently testing how nucleosome positioning affects gene expression using a human factor IX (hFIX) cDNA gene driven by the human elongation factor 1 alpha (EF-1 α) promoter as a model vector. Putative nucleosome positioning signals, including the Widom 601 sequence (a reported synthetic positioning signal), a CTG₍₁₂₀₎ repeat, and portions of the hFIX intron I were individually added at strategic locations in the EF-1 α promoter. We delivered these constructs into the livers of B6 mice by hydrodynamic tail vein injection and also into ME-180 cervical cancer cells in culture. hFIX levels were quantified by enzyme-linked immunosorbent assays (ELISAs).

To further elucidate the course of chromatin modulation, we are mapping the nucleosome positions within the vector DNA isolated from mouse liver and tissue culture cells. Historically, this has been attempted using low-resolution methods such as Southern blots, but we have developed a DNA hybridization method that allows for high-resolution, direct evidence of nucleosome positioning. By using a biotin-labeled segment of the parent vector as a probe, we have pulled out nucleosome core DNA collected from tissues. Through Sanger sequencing, we have found strongly positioned nucleosomes in silenced plasmids and will also use deep sequencing platforms to identify nucleosome positions within the vector.

In the future, this work could improve gene therapy applications by supplying broadly applicable guidelines for how to position nucleosomes to sustain expression of a delivered gene, regardless of the vector type.



R. K. FUNK



L. E. GRACEY

WEDNESDAY
ROOM B

10:30 A.M.

Analyzing variation in gene regulation in humans: global analysis of NF- κ B binding using Chip-Seq in different individuals**MAYA KASOWSKI**, Yale University School of Medicine

Mentor: Michael Snyder, Ph.D., Yale University 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 am mapping the binding sites of a key regulator of the immune system, NF- κ B, and Pol II in eight HapMap lymphoblastoid cell lines of African, European, and Asian ancestry. Divergence in NF- κ B binding may help explain dif-

ferences in the response to pathogens, inflammatory processes, and the progression of certain cancers. We also plan to measure gene expression in each cell line following TNF- α stimulation with Affymetrix GeneChips in order to correlate differences in binding profiles with expression.

We have completed the preparation of Pol II Chip DNA and have run single replicates for eight samples on Illumina sequencers. Inspection of the signal tracks suggests that most targets are the same, but some differences have been observed. To cover all possible targets, we are aiming for approximately 9 million mapped reads generated from three biological replicates and then will systematically analyze the data. Then we will generate similar results for NF- κ B.

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.



M. KASOWSKI

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INDEX OF PRESENTATION TIMES

Name	Day	Time	Room	Page
Adler, Joel T.	Tuesday	9:15 a.m.	Auditorium	62
Akhtar, Amana	Tuesday	5:30–6:15 p.m.	Cloister	68
Arboleda, Valerie A.	Tuesday	5:30–6:15 p.m.	Cloister	69
Aronson, Joshua Paul	Monday	9:00 a.m.	B	28
Arora, Amrita	Tuesday	5:30–6:15 p.m.	Cloister	68
Attenello, Frank Joseph	Monday	2:45 p.m.	B	33
Baron, Jason M.	Monday	10:15 a.m.	A	22
Batra, Sonny	Monday	3:30–4:15 p.m.	Atrium	45
Beaudry, Steven	Monday	1:45 p.m.	A	24
Bennett, Christina	Wednesday	9:15 a.m.	B	79
Berg, Kristin Marie	Monday	4:15–5:00 p.m.	Atrium	50
Bhan, Irun	Monday	4:15–5:00 p.m.	Atrium	51
Boonyaratanakornkit, Jim B.	Monday	2:00 p.m.	A	24
Brauer, Steven	Monday	9:15 a.m.	A	20
Brezing, Christina Ann	Tuesday	6:15–7:00 p.m.	Cloister	72
Caballero, Jorge A.	Monday	9:00 a.m.	Auditorium	35
Campion, Paul	Monday	3:00 p.m.	B	34
Caretto, David Christopher	Monday	9:30 a.m.	A	21
Chao, Mark P.	Monday	9:45 a.m.	Auditorium	36
Choe, Jessica S.	Monday	2:00 p.m.	B	32
Choi, Lynn Y.	Monday	9:15 a.m.	B	28
Chou, David	Monday	9:00 a.m.	A	20
Cohen, Jonah	Monday	2:15 p.m.	Auditorium	40
Cushing, Kelly	Tuesday	6:15–7:00 p.m.	Cloister	71
Dalai, Sudeb C.	Monday	4:15–5:00 p.m.	Atrium	47
DeCaro, John Joseph	Tuesday	6:15–7:00 p.m.	Cloister	70
Dickman, Katherine	Monday	2:30 p.m.	A	25
Dingman, Andra L.	Tuesday	6:15–7:00 p.m.	Cloister	74
Dong, Fei	Tuesday	8:30 a.m.	Auditorium	61
Durrani, Amir Kahn	Wednesday	10:15 a.m.	A	78
Edlin, Rachel S.	Tuesday	9:00 a.m.	A	54
Ehsanian, Reza	Monday	2:30 p.m.	Auditorium	40
Ellens, Damien J.	Tuesday	9:30 a.m.	B	59
Ene, Chiba	Tuesday	9:45 a.m.	Auditorium	63
Fenster, Robert Jonathan	Monday	1:30 p.m.	B	31
Funk, Ryan Kevin	Wednesday	10:00 a.m.	B	81
Garber, Haven	Monday	4:15–5:00 p.m.	Atrium	50
Gehrie, Eric	Monday	9:45 a.m.	A	21
Giacomini, Craig P.	Monday	3:30–4:15 p.m.	Atrium	44
Gordon, Joshua A.	Wednesday	9:30 a.m.	A	77

INDEX OF PRESENTATION TIMES

Name	Day	Time	Room	Page
Gracey, Lia EunHee	Wednesday	10:15 a.m.	B	81
Gupta, Rohit	Tuesday	5:30–6:15 p.m.	Cloister	66
Hakkinen, Kirsi	Tuesday	5:30–6:15 p.m.	Cloister	65
Hall, Julie M.	Tuesday	6:15–7:00 p.m.	Cloister	72
Han, Ian Carlos	Monday	4:15–5:00 p.m.	Atrium	52
Hanisch, Jesse J.	Wednesday	9:30 a.m.	B	80
Harrison, Victor Sanoe	Tuesday	5:30–6:15 p.m.	Cloister	67
Hoang, Stanley	Tuesday	6:15–7:00 p.m.	Cloister	74
Hoover, Andrew C.	Monday	2:45 p.m.	Auditorium	41
Hsu, Kimberly	Tuesday	9:45 a.m.	B	59
Hsu, Mark	Monday	4:15–5:00 p.m.	Atrium	52
Huang, Susan Jen	Tuesday	10:00 a.m.	B	60
Jaetao, Jason E.	Monday	3:30–4:15 p.m.	Atrium	42
Jagger, Brett	Monday	2:15 p.m.	A	25
Jimenez, Cynthia Ann	Monday	10:15 a.m.	Auditorium	37
Jiramongkolchai, Kim	Tuesday	8:45 a.m.	A	53
Kaneko, Osamu Fernando	Monday	3:30–4:15 p.m.	Atrium	44
Kanjilal, Sanjat	Monday	10:15 a.m.	B	30
Kasowski, Maya	Wednesday	10:30 a.m.	B	82
Kern, Adam	Monday	9:15 a.m.	Auditorium	35
Khan, M. Ali	Monday	10:30 a.m.	Auditorium	38
Kim, Charles	Tuesday	9:15 a.m.	A	54
Kim, Somi	Tuesday	10:00 a.m.	A	56
Koeth, Robert A.	Wednesday	9:00 a.m.	A	76
Lee, Amie Yoo-Youn	Tuesday	8:30 a.m.	B	57
Lee, Darrin J.	Tuesday	9:15 a.m.	B	58
Lee, Jung Eun	Monday	10:30 a.m.	A	23
Lee, Michael Sangmin	Monday	3:30–4:15 p.m.	Atrium	43
Lew, Quan Lan Jasmine	Wednesday	9:00 a.m.	B	79
Li, Loretta S.	Monday	9:30 a.m.	Auditorium	36
Lo, Sheng-fu	Monday	3:15 p.m.	A	27
Longmire, Michelle Rae	Tuesday	9:45 a.m.	A	55
Malhotra, Ajay	Monday	3:30–4:15 p.m.	Atrium	42
Mawad, Kareem	Tuesday	6:15–7:00 p.m.	Cloister	75
McPoland, Paula	Tuesday	9:30 a.m.	Auditorium	63
Meoli, Elise M.	Monday	4:15–5:00 p.m.	Atrium	49
Mikell, Charles	Tuesday	9:00 a.m.	B	58
Milner, Eric	Tuesday	6:15–7:00 p.m.	Cloister	73
Minder, Camille Michael	Tuesday	5:30–6:15 p.m.	Cloister	67
Minear, Steve	Wednesday	9:45 a.m.	A	77

INDEX OF PRESENTATION TIMES

Name	Day	Time	Room	Page
Mong, Sandy	Monday	10:00 a.m.	Auditorium	37
Muñoz, Eric C.	Tuesday	8:45 a.m.	B	57
Narendra, Derek Paul	Monday	1:45 p.m.	B	31
Nguyen, Anvy	Monday	4:15–5:00 p.m.	Atrium	48
Nguyen, Giang Huong	Monday	1:45 p.m.	Auditorium	39
Orina, Josiah N.	Monday	3:30–4:15 p.m.	Atrium	46
Palavalli, Lavanya H.	Tuesday	6:15–7:00 p.m.	Cloister	70
Parekh, Krishna	Tuesday	9:00 a.m.	Auditorium	62
Parikh, Tanvi	Monday	4:15–5:00 p.m.	Atrium	51
Patel, Mrinali	Wednesday	9:15 a.m.	A	76
Patel, Nishant Dinesh	Tuesday	6:15–7:00 p.m.	Cloister	71
Patel, Priyesh A.	Tuesday	5:30–6:15 p.m.	Cloister	66
Perez, Bradford	Monday	3:30–4:15 p.m.	Atrium	46
Pricola, Katie L.	Tuesday	5:30–6:15 p.m.	Cloister	65
Quick, John S.	Monday	3:30–4:15 p.m.	Atrium	45
Rao, Abigail	Tuesday	6:15–7:00 p.m.	Cloister	75
Rickert, Charles	Monday	3:30–4:15 p.m.	Atrium	43
Russo, Andrea L.	Tuesday	10:00 a.m.	Auditorium	64
Schulman, Joshua	Tuesday	9:30 a.m.	A	55
Schweitzer, Andrew D.	Monday	10:00 a.m.	B	30
Sinha, Shashank S.	Monday	9:30 a.m.	B	29
Snyder, Alexandra	Monday	1:30 p.m.	A	23
Steward-Tharp, Scott	Monday	2:45 p.m.	A	26
Swisher, Matthew	Tuesday	5:30–6:15 p.m.	Cloister	69
Sworder, Brian	Wednesday	10:00 a.m.	A	78
Thaker, Ashesh	Tuesday	8:45 a.m.	Auditorium	61
Toprani, Sheela	Monday	9:45 a.m.	B	29
Ungaro, Ryan	Monday	3:00 p.m.	A	26
Verduzco, Luis A.	Tuesday	6:15–7:00 p.m.	Cloister	73
Viani, Kyle	Monday	4:15–5:00 p.m.	Atrium	48
Weiner, Shoshana	Monday	1:30 p.m.	Auditorium	38
Whitlock, Elizabeth L.	Monday	2:30 p.m.	B	33
Wirka, Robert	Wednesday	9:45 a.m.	B	80
Wong, Ambrose Hon Wai	Monday	4:15–5:00 p.m.	Atrium	47
Wysong, Ashley	Monday	2:00 p.m.	Auditorium	39
Yonekura Collier, Ai-ris	Tuesday	10:15 a.m.	B	60
Yu, Charles Q.	Monday	2:15 p.m.	B	32
Zaid, Harras	Monday	10:00 a.m.	A	22
Zambricki, Elizabeth A.	Monday	4:15–5:00 p.m.	Atrium	49
Zwang, Nicholas A.	Tuesday	8:30 a.m.	A	53

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